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(71) Applicants (for all designated States except US): **THE UNIVERSITY OF TENNESSEE RESEARCH FOUNDATION** [US/US]; 1534 White Avenue-Suite 403, Knoxville, TN 37996-1527 (US). **COLORADO STATE UNIVERSITY RESEARCH FOUNDATION** [US/US]; P.O. Box 483, Fort Collins, CO 80522 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **LEE, Richard, E.**

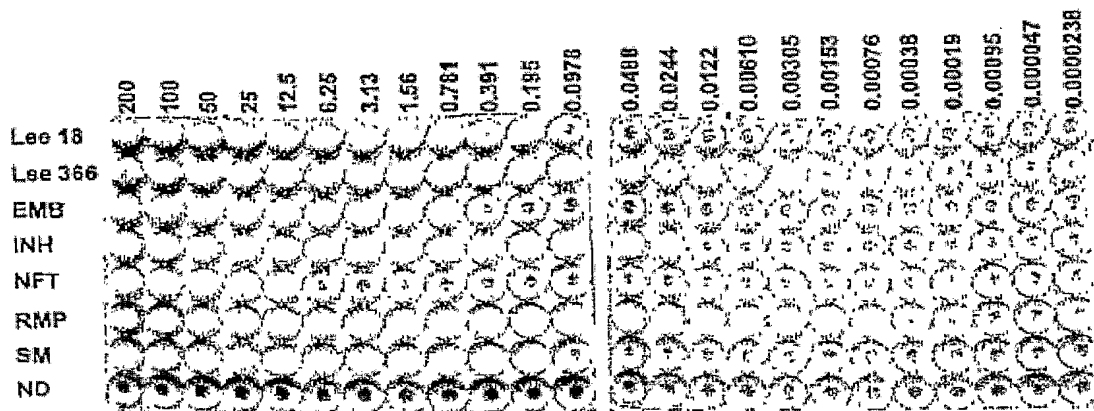
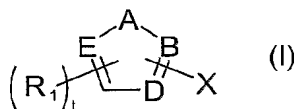
[US/US]; 8233 Byre Hollow Cave, Cordova, TN 38018 (US). **TANGALLAPALLY, Rajendra, Prasad** [IN/US]; Department of Pharmaceutical Science, 847 Monroe Ave - Room 327, Memphis, TN 38163 (US). **MCNEIL, Michael, R.** [US/US]; 6801 N County Road-15, Fort Collins, CO 80524 (US). **LENAERTS, Anne** [BE/US]; 4230 Goldeneye Drive, Fort Collins, CO 80526 (US).

(74) Agent: **TAYLOR, Arles, A., Jr.**; Jenkins, Wilson & Taylor, P.A., Suite 1400 University Tower, 3100 Tower Boulevard, Durham, NC 27707 (US).

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(54) Title: HETEROCYCLIC AMIDES WITH ANTI-TUBERCULOSIS ACTIVITY



(57) Abstract: Compounds having the general structure (I) : wherein A is selected from the group consisting of oxygen, sulfur, and NR<sub>15</sub>, and R<sub>15</sub> is selected from the group consisting of H, alkyl, aryl, substituted alkyl, and substituted aryl; B, D, and E are each independently selected from the group consisting of CH, nitrogen, sulfur and oxygen; R<sub>1</sub> is selected from the group consisting of nitro, halo, alkyl ester, phenylsulfanyl, phenylsulfinyl, phenylsulfonyl and sulfonic acid; t is an integer from 1 to 3; and X is a substituted amide and methods of using the novel compounds for treating infections caused microorganisms, including *Mycobacterium tuberculosis*.



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## DESCRIPTION

### HETEROCYCLIC AMIDES WITH ANTI-TUBERCULOSIS ACTIVITY

#### RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/487,004, filed July 14, 2003 and U.S. Provisional Patent Application Serial No. 60/551,409, filed March 9, 2004; the disclosures of both of which are incorporated herein by reference in their entireties.

#### GOVERNMENT INTEREST

This invention was made in part with support from grant numbers AI-053796 and NO1-AI-95385 from the National Institutes of Health. The United States government has certain rights in this invention.

#### TECHNICAL FIELD

The presently disclosed subject matter relates to methods of combating microbial infections with novel amides. More particularly, the presently disclosed subject matter relates to novel amide compounds and methods of combating microbial infections caused by *Mycobacterium tuberculosis* using the novel compounds.

#### ABBREVIATIONS

Ag.	=	aqueous
Boc	=	t-butyloxycarbonyl
CAT	=	catalyst
CDCl <sub>3</sub>	=	deuterated chloroform
CPBA	=	chloroperoxybenzoic acid
DEAD	=	diethyl azodicarboxylate
dil.	=	dilute
DMAP	=	4-dimethylaminopyridine
DMF	=	dimethyl formamide
DMSO	=	dimethylsulfoxide
EI	=	electron-impact ionization

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	EMS	=	Ethambutyl
	ESI	=	electrospray ionization
	Et	=	ethyl
	EtOAc	=	ethyl acetate
5	Glf	=	UDP-galactose mutase
	HIV	=	human immunodeficiency virus
	HPLC	=	high-performance liquid chromatography
	Hz	=	hertz
	IC <sub>50</sub>	=	Inhibitory concentration
10	INH	=	isoniazid
	J	=	spin coupling constant
	kg	=	kilogram
	KHMDS	=	potassium hexamethyldisilazide
	<i>M. tuberculosis</i>	=	<i>Mycobacterium tuberculosis</i>
15	MDRTB	=	multidrug resistant tuberculosis
	μg	=	microgram
	mg	=	milligram
	MHz	=	megahertz
	MIC	=	minimum inhibitory concentration
20	mL	=	milliliter
	mM or mmol	=	millimolar
	MTD	=	maximum tolerated dose
	NE	=	no drug
	NET	=	nitrofurantoin
25	nm	=	nanometer
	NMR	=	nuclear magnetic resonance
	Oac	=	acetate
	OD	=	optical density
	pet. ether	=	petroleum ether
30	Py	=	pyridine

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	Red-Al	=	sodium bis(2-methoxyethoxy)aluminum hydride
	RMP	=	rifampin
	SM	=	streptomycin sulfate
5	solu.	=	solution
	TB	=	tuberculosis
	TFA	=	trifluoroacetic acid
	THF	=	tetrahydrofuran
	TLC	=	thin layer chromatography
10	UV	=	ultraviolet

### BACKGROUND ART

The global burden of tuberculosis (TB) is immense. In 1997 there were an estimated 7.96 million new and 16.2 million existing cases with the worldwide mortality rate at 23%. HIV infection is a key risk factor in TB reactivation rates. HIV infected patients have an elevated risk of primary or reactivated TB, and such active infectious process may enhance HIV replication and increase risk of death. An additional major concern is the rise of multidrug resistant tuberculosis (MDRTB). No new effective treatments have been developed since the introduction of Rifampin in 1971, even though there have been significant advances in drug development technologies. Consequently there is an urgent need to develop new, potent, fast acting anti-tuberculosis drugs with low toxicity profiles. There is also a need to develop anti-tuberculosis drugs that can be used in conjunction with drugs used to treat HIV infections.

The mycobacterial cell wall is a complex and intriguing mixture of unique components, which sets mycobacteria apart from all other known bacteria. Many of the tuberculosis bacilli characteristics, such as its relatively small size, the ability to grow in macrophages, drug resistance and hydrophilicity are believed to result from components within ultrastructure of the cell wall. Since many of the structural components of the cell wall are not found in humans,

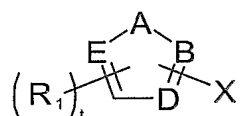
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enzymes involved in cell wall biosynthesis have proved to be a very fertile ground for the development of anti-tuberculosis drugs. Current anti-tuberculosis drugs such as isoniazid, ethionamide and ethambutol are all believed to act against mycobacterial cell wall biosynthesis, validating the enzymes of cell wall biosynthesis as targets for further drug development.

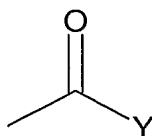
Thus, there continues to be a need for improvement in the art for additional compounds having desirable anti-microbial activity, whether against the representative pathogens referenced above or against other pathogens. Of particular interest would be compounds having activity in the treatment of human tuberculosis, an infectious disease for which new treatments for multidrug resistant organisms associated with the disease are a particular need in the art.

#### SUMMARY

The presently disclosed subject matter provides compounds having the following general structure:



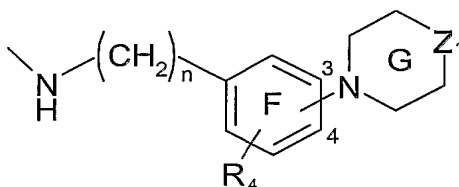
wherein A is selected from the group consisting of oxygen, sulfur, and NR<sub>15</sub>, wherein R<sub>15</sub> is selected from the group consisting of H, alkyl, aryl, substituted alkyl, and substituted aryl; B, D, and E are each independently selected from the group consisting of CH, nitrogen, sulfur and oxygen; R<sub>1</sub> is selected from the group consisting of nitro, halo, alkyl ester, phenylsulfanyl, phenylsulfinyl, phenylsulfonyl and sulfonic acid; t is an integer from 1 to 3; and X is a substituted amide. In some embodiments, "X" has the following general structure:



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wherein Y is a substituted amine. In some embodiments, Y is an aromatic monoamine. In some embodiments, Y has the general formula  $-NR_2R_3$ , and  $R_2$  and  $R_3$  are each independently selected from the group consisting of H, alkyl, aryl, substituted alkyl, and substituted aryl, or  $R_2$  and  $R_3$  together form a ring structure with the nitrogen atom to which they are attached.

In some embodiments, Y comprises the formula:



wherein:

$n$  is an integer from 0 to 8;  
 $R_4$  is selected from the group consisting of hydrogen, halo, alkyl, substituted alkyl, and alkoxy; and

$Z_1$  is selected from the group consisting of oxygen, sulfur, sulfoxide,

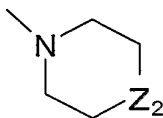
sulfone,  $NR_5$ , and  $\begin{array}{c} R_6 \\ \diagup \\ C \\ \diagdown \\ R_7 \end{array}$ , wherein  $R_5$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkoxy, carbonyl, aryl, substituted aryl, and  $-(C=O)-NR_8R_9$ ; wherein  $R_6$  and  $R_7$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl, and  $R_8$  and  $R_9$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl.

In some embodiments,  $n$  is zero. In some embodiments,  $n$  is one. In some embodiments, ring G is in the 3-position of ring F. In some embodiments, ring G is in the 4-position of ring F. In some embodiments,  $Z_1$  is oxygen or sulfur. In some embodiments,  $Z_1$  comprises  $NR_5$ . In some

embodiments,  $Z_1$  comprises  $\begin{array}{c} R_6 \\ \diagup \\ C \\ \diagdown \\ R_7 \end{array}$ .

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In some embodiments, Y comprises the formula:



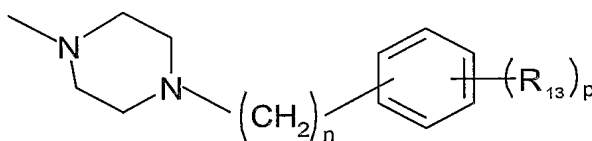
wherein:

- 5  $Z_2$  is selected from the group consisting of oxygen,  $NR_{10}$ , and  $\begin{array}{c} R_{11} \\ \diagup \\ C \\ \diagdown \\ R_{12} \end{array}$ ,  $R_{10}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl, and  $R_{11}$  and  $R_{12}$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl.

In some embodiments,  $Z_2$  comprises  $NR_{10}$ . In some embodiments,  $Z_2$

- 10 comprises  $\begin{array}{c} R_{11} \\ \diagup \\ C \\ \diagdown \\ R_{12} \end{array}$ .

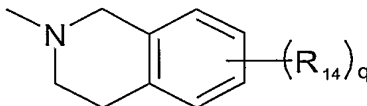
In some embodiments, Y comprises the formula:



wherein:

- 15  $n$  is an integer from 0 to 8;  
 $p$  is an integer from 1 to 5; and  
 $R_{13}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl.
- 20 In some embodiments,  $n$  is zero. In some embodiments,  $n$  is one. In some embodiments,  $R_{13}$  comprises a substituted alkyl group. In some embodiments, the alkyl group substituent comprises an aryl group.

In some embodiments, Y comprises the formula:

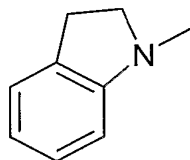




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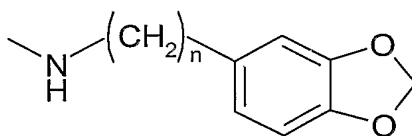
wherein  $q$  is an integer from 1 to 4; and  $R_{14}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl.

In some embodiments, Y comprises:



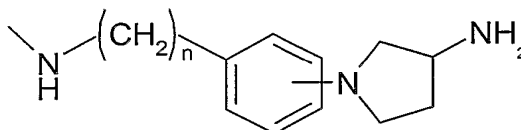
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In some embodiments, Y comprises the formula:



wherein  $n$  is an integer from 0 to 8.

In some embodiments, Y comprises the formula:



10

wherein  $n$  is an integer from 0 to 8.

The presently disclosed subject matter further describes methods of using the novel compounds disclosed herein. In some embodiments, the presently disclosed subject matter comprises methods of killing or inhibiting the growth of a microorganism comprising contacting the microorganism with an effective amount of one or more of the novel compounds. In some  
15  
embodiments, the microorganism is a member of the genus *Mycobacterium*. More particularly, in some embodiments, the microorganism is *Mycobacterium tuberculosis*.

In some embodiments, the presently disclosed subject matter comprises  
20  
methods of treating a microbial infection in a subject comprising administering a therapeutically effective amount of one or more of the novel compounds disclosed herein. In some embodiments, the microbial infection is caused by a member of the genus *Mycobacterium*. More particularly, in some embodiments, the member is *Mycobacterium tuberculosis*.

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5 The presently disclosed subject matter further encompasses pharmaceutical formulations for the treatment of tuberculosis comprising one or more novel compounds disclosed herein in a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical formulation is acceptable for intravenous administration and/or oral administration.

10 It is accordingly an object of the presently disclosed subject matter to provide compounds that are useful in the treatment of microbial infections. It is another object of the invention to provide pharmaceutical formulations for use in the treatment of microbial infections. It is still another object of the invention to provide methods for treating microbial infections.

15 Some of the objects of the presently disclosed subject matter having been stated hereinabove, and which are addressed in whole or in part by the presently disclosed subject matter, other objects will become evident as the description proceeds when taken in connection with the accompanying drawings as best described hereinbelow.

#### BRIEF DESCRIPTION OF THE DRAWINGS

20 Figure 1 shows MIC data for two nitrofuranyl amides, 4 classical TB drugs, and nitrofurantoin. Abbreviations; ethambutol (EMS), isoniazid (INH), nitrofurantoin (NET), rifampin (RMP), streptomycin sulfate (SM), no drug (NE)). Drugs were serially diluted two-fold across 24 wells. The concentrations are reported in  $\mu\text{g/ml}$  and are shown above each column.

Figure 2 shows a summary of the preliminary nitrofuranyl amide series development.

25 Figure 3 shows alternative heterocyclic novel compounds disclosed herein.

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## DETAILED DESCRIPTION OF THE INVENTION

The presently disclosed subject matter will now be described more fully hereinafter with reference to the accompanying Examples, in which preferred embodiments are shown. The presently disclosed subject matter can, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the embodiments to those skilled in the art.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this presently described subject matter belongs. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

Throughout the specification and claims, a given chemical formula or name shall encompass all optical and stereoisomers, as well as racemic mixtures where such isomers and mixtures exist.

I. Definitions

The term "independently selected" is used herein to indicate that the R groups, e.g., R<sub>2</sub>, and R<sub>3</sub> can be identical or different (e.g., R<sub>2</sub> and R<sub>3</sub> may both be substituted alkyls, or R<sub>2</sub> may be hydrogen and R<sub>3</sub> may be a substituted aryl, etc.).

A named "R", "X" or "Y" group will generally have the structure that is recognized in the art as corresponding to a group having that name, unless specified otherwise herein. For the purposes of illustration, certain representative "R," "X" and "Y" groups as set forth above are defined below. These definitions are intended to supplement and illustrate, not preclude, the definitions known to those of skill in the art.

As used herein, the term "acyl" refers to an organic acid group wherein the -OH of the carboxyl group has been replaced with another substituent (i.e., as represented by RCO—, wherein R is an alkyl or an aryl group as defined

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herein). As such, the term "acyl" specifically includes arylacyl groups. Specific examples of acyl groups include acetyl and benzoyl.

"Acylamino" refers to an acyl-NH-- group wherein acyl is as previously described.

5 "Acyloxy" as used herein refers to an acyl-O-- group wherein acyl is as previously described.

As used herein, the term "alkyl" means C<sub>1</sub>-C<sub>20</sub> inclusive (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms), linear, branched, or cyclic, saturated or unsaturated (i.e., alkenyl and alkynyl) hydrocarbon chains, including for example, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl, octyl, ethenyl, propenyl, butenyl, pentenyl, hexenyl, octenyl, butadienyl, propynyl, butynyl, pentynyl, hexynyl, heptynyl, and allenyl groups.

15 The alkyl group can be optionally substituted (a "substituted alkyl") with one or more alkyl group substituents which can be the same or different, where "alkyl group substituent" includes alkyl, halo, arylamino, acyl, hydroxy, aryloxy, alkoxy, alkylthio, aryl, arylthio, aralkyloxy, aralkylthio, carboxy, alkoxycarbonyl, oxo, ester and cycloalkyl. Representative substituted alkyls include, for example, benzyl, trifluoromethyl and the like. There can be optionally inserted  
20 along the alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, alkyl (also referred to herein as "alkylaminoalkyl"), or aryl. "Branched" refers to an alkyl group in which an alkyl group, such as methyl, ethyl or propyl, is attached to a linear alkyl chain.

25 "Alkoxy" or "alkoxyalkyl" as used herein refer to an alkyl-O-- group wherein alkyl is as previously described. The term "alkoxy" as used herein can refer to C<sub>1-20</sub> inclusive (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms), linear, branched, or cyclic, saturated or unsaturated oxo-hydrocarbon chains, including, for example, methoxyl, ethoxyl, propoxyl, isopropoxyl, butoxyl, t-butoxyl, and pentoxyl.  
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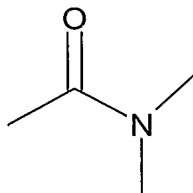
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"Alkoxy carbonyl" as used herein refers to an alkyl-O--CO-- group. Exemplary alkoxy carbonyl groups include methoxy carbonyl, ethoxy carbonyl, butyloxy carbonyl, and t-butyloxy carbonyl (Boc).

5 "Alkyl carbamoyl" as used herein refers to a R'RN--CO-- group wherein one of R and R' is hydrogen and the other of R and R' is alkyl as described herein.

"Alkylene" refers to a straight or branched bivalent aliphatic hydrocarbon group having from 1 to about 20 carbon atoms, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. The alkylene group can be straight, branched or cyclic. The alkylene group can be also optionally be  
10 unsaturated and/or substituted with one or more "alkyl group substituents." There can be optionally inserted along the alkylene group one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms (also referred to herein as "alkylaminoalkyl"), wherein the nitrogen substituent is alkyl as previously  
15 described. Exemplary alkylene groups include methylene (--CH<sub>2</sub>--); ethylene (--CH<sub>2</sub>-CH<sub>2</sub>--); propylene (--(CH<sub>2</sub>)<sub>3</sub> --); cyclohexylene (--C<sub>6</sub>H<sub>10</sub> --); --CH=CH--CH=CH--; --CH=CH--CH<sub>2</sub>--; --(CH<sub>2</sub>)<sub>q</sub>--N(R)--(CH<sub>2</sub>)<sub>r</sub>--, wherein each of q and r is independently an integer from 0 to about 20, e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, and R is hydrogen or lower alkyl;  
20 methylenedioxy (--O--CH<sub>2</sub>--O--); and ethylenedioxy (--O--(CH<sub>2</sub>)<sub>2</sub>--O--). An alkylene group can have about 2 to about 3 carbon atoms and can further have 6-20 carbons.

As used herein, the term "amide" refers to a group having the amide functional group:



25

The amide group can be optionally substituted (a "substituted amide") with one or more amide group substituents which can be the same or different, where "amide group substituent" includes but is not limited to alkyl, halo,

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arylamino, acyl, hydroxy, aryloxy, alkoxyl, alkylthio, aryl, arylthio, aralkyloxy, aralkylthio, carboxy, alkoxycarbonyl, oxo, ester and cycloalkyl. In some embodiments, the amide group is substituted as described in detail below and throughout the specification, including the Examples and claims.

5           The terms "amine" and "amino" are used herein to refer to the group --NZ<sub>1</sub>Z<sub>2</sub>, where each of Z<sub>1</sub> and Z<sub>2</sub> is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, such as phenylamine (aniline), and methoxyaniline  
10           (anisidine), alkoxyl, aryloxy, silyl, furfuryl, and combinations thereof. Additionally, the amine or amino group may be represented as N<sup>+</sup> Z<sub>1</sub> Z<sub>2</sub> Z<sub>3</sub>, i.e., a quaternary nitrogen group, with the previous definitions applying, and Z<sub>3</sub> being one of H and alkyl. Thus, substituted and unsubstituted amines of the compounds described herein may be primary amines, secondary amines,  
15           tertiary amines or quaternary ammonium salts.

          As used herein, the term "aniline" refers to phenylamine.

          As used herein, the term "anisidine" refers to a phenyl group substituted with an amine at one carbon atom and a methyl ether at another carbon atom, e.g. 2-methoxyaniline, 4-methoxyaniline, etc.

20           As used herein, the term "aralkyl" refers to an aryl-alkyl- group wherein aryl and alkyl are as previously described. Exemplary aralkyl groups include benzyl, phenylethyl, and naphthylmethyl.

          "Aroylamino" refers to an aroyl-NH—group, wherein aroyl is defined as an acyl radical derived from an aromatic carboxylic acid, i.e. an arylcarbonyl  
25           substituent group, such as benzoyl.

          The term "aryl" is used herein to refer to an aromatic substituent which may be a single aromatic ring, or multiple aromatic rings that are fused together, linked covalently, or linked to a common group, such as a methylene or ethylene moiety. The common linking group may also be a carbonyl, as in  
30           benzophenone, or oxygen, as in diphenylether, or nitrogen, as in diphenylamine. The term "aryl" specifically encompasses heterocyclic aromatic

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compounds. The aromatic ring(s) may comprise phenyl, naphthyl, biphenyl, diphenylether, diphenylamine and benzophenone, among others. In some embodiments, the term "aryl" means a cyclic aromatic comprising about 5 to about 10 carbon atoms, e.g. 5, 6, 7, 8, 9, or 10 carbon atoms, and including 5 and 6-membered hydrocarbon and heterocyclic aromatic rings.

The aryl group can be optionally substituted (a "substituted aryl") with one or more aryl group substituents which can be the same or different, where "aryl group substituent" includes alkyl, aryl, aralkyl, hydroxyl, alkoxyl, aryloxy, aralkoxy, carboxy, acyl, halo, nitro, alkoxycarbonyl, aryloxy carbonyl, aralkoxycarbonyl, acyloxy, acylamino, aroylamino, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, arylthio, alkylthio, alkylene and  $-NR'R''$ , where  $R'$  and  $R''$  can be each independently hydrogen, alkyl, aryl and aralkyl.

Specific examples of aryl groups include but are not limited to cyclopentadienyl, phenyl, furan, thiophene, pyrrole, pyran, pyridine, imidazole, isothiazole, isoxazole, pyrazole, pyrazine, triazine, pyrimidine, quinoline, isoquinoline, indole, and the like.

"Aralkoxycarbonyl" as used herein refers to an aralkyl-O--CO-- group. An exemplary aralkoxycarbonyl group is benzyloxycarbonyl.

"Aryloxy carbonyl" as used herein refers to an aryl-O--CO—group. Exemplary aryloxy carbonyl groups include phenoxy- and naphthoxy-carbonyl.

As used herein, the term "Aryloxy" refers to an aryl-O-- group wherein the aryl group is as previously described. The term "aryloxy" as used herein can refer to phenyloxy or hexyloxy, and alkyl, halo, or alkoxyl substituted phenyloxy or hexyloxy.

"Carbamoyl" as used herein refers to an  $H_2N$ --CO—group.

As used herein, the terms "Cyclic" and "cycloalkyl" refer to a non-aromatic mono- or multicyclic ring system of about 3 to about 10 carbon atoms, e.g., 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. The cycloalkyl group can be optionally partially unsaturated. The cycloalkyl group can be also optionally substituted with an alkyl group substituent as defined herein, oxo, and/or alkylene. There can be optionally inserted along the cyclic alkyl chain one or

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more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, lower alkyl, or aryl, thus providing a heterocyclic group. Representative monocyclic cycloalkyl rings include cyclopentyl, cyclohexyl, and cycloheptyl. Multicyclic cycloalkyl rings include adamantyl, octahydronaphthyl, decalin, camphor, camphane, and noradamantyl.

The term "carbonyl" as used herein refers to the  $-(C=O)-$  group.

The term "carboxyl" as used herein refers to the  $-COOH$  group.

"Dialkylcarbamoyl" as used herein refers to  $R'RN-CO-$  group wherein each of R and R' is independently alkyl as previously described.

The term "halo" is defined as being selected from the group consisting of Br, Cl, I and F.

The term "hydroxyl" as used herein refers to the  $-OH$  group.

The term "hydroxyalkyl" as used herein refers to an alkyl group substituted with an  $-OH$  group.

The term "furfuryl" as used herein refers to the group comprised of a methyl furan radical.

The term "nitrile" as used herein refers to the  $-C\equiv N$  group.

The term "nitro" is defined as the functional group  $-NO_2$ .

The term "oxo" as used herein refers to a compound wherein a carbon atom is replaced by an oxygen atom.

The term "phenylsulfanyl" refers to a substituent group having the general formula  $Ar-S-$ , wherein Ar is an aryl group as defined herein.

The term "phenylsulfinyl" refers to a substituent group having the general formula  $Ar-S(=O)-$ , wherein Ar is an aryl group as defined herein and  $S(=O)$  represents an oxygen atom bound to the sulfur atom through a double bond.

The term "phenylsulfonyl" refers to a substituent group having the general formula  $Ar-S(=O)_2-$ , wherein Ar is an aryl group as defined herein and  $S(=O)_2$  represents two oxygen atoms which are each bound to the sulfur atom through a double bond.

The term "sulfonic acid" refers to a compound comprising the functional



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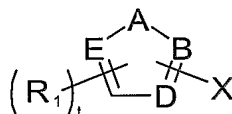
group  $R-S(=O)_2OH$ , wherein R is alkyl or aryl as defined herein and  $S(=O)_2$  represents two oxygen atoms which are each bound to the sulfur atom through a double bond.

5 The term "thio" as used herein refers to a compound wherein a carbon or oxygen atom is replaced by a sulfur atom.

## II. Novel Compounds

### A. Representative Embodiments

Described herein is a compound of the formula:

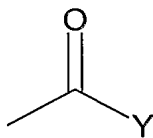


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wherein A is selected from the group consisting of oxygen, sulfur, and  $NR_{15}$ , and  $R_{15}$  is selected from the group consisting of H, alkyl, aryl, substituted alkyl, and substituted aryl; B, D, and E are each independently selected from the group consisting of CH, nitrogen, sulfur and oxygen;  $R_1$  is selected from the group consisting of nitro, halo, alkyl ester, phenylsulfanyl, phenylsulfinyl, phenylsulfonyl and sulfonic acid; t is an integer from 1 to 3 (e.g., 1, 2 or 3); and X is a substituted amide. In particular embodiments,  $R_1$  is nitro.

15

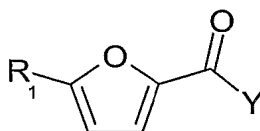
In some embodiments, X has the formula:



20

wherein Y is a substituted amine. In particular embodiments, Y is an aromatic monoamine.

In some embodiments, the novel compounds are defined as having a formula as follows:



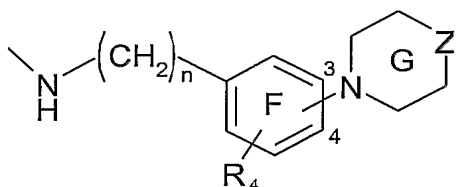
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wherein  $R_1$  is selected from the group consisting of halo, nitro, alkyl ester, phenylsulfanyl, phenylsulfinyl, phenylsulfonyl and sulfonic acid; and Y is a substituted amine.

In some embodiments of the novel compounds, Y can be:

- 5 (a)  $-NR_2R_3$ , wherein  $R_2$  and  $R_3$  are each independently selected from the group consisting of H, alkyl, aryl, substituted alkyl, and substituted aryl, or  $R_2$  and  $R_3$  together form a ring structure with the nitrogen atom to which they are attached;

(b)

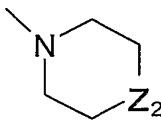


10 wherein n is an integer from 0 to 8 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, or 8);  $R_4$  is selected from the group consisting of hydrogen, halo, alkyl, substituted alkyl, and alkoxy; and  $Z_1$  is selected from the group

consisting of oxygen, sulfur, sulfoxide, sulfone,  $NR_5$ , and  $\begin{array}{c} R_6 \\ \diagup \\ C \\ \diagdown \\ R_7 \end{array}$ ;

15 wherein  $R_5$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkoxy, carbonyl, aryl, substituted aryl, and  $-(C=O)-NR_8R_9$ , wherein  $R_6$  and  $R_7$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl and  $R_8$  and  $R_9$  are each independently  
20 selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl;

(c)



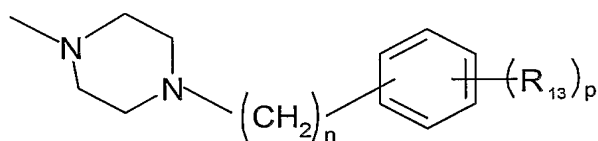
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wherein  $Z_2$  is selected from the group consisting of oxygen,  $NR_{10}$ ,

and  $\begin{array}{c} \text{C} - R_{11} \\ \diagdown \\ R_{12} \end{array}$ ;  $R_{10}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl; and  $R_{11}$  and  $R_{12}$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl;

5

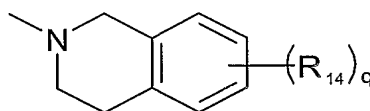
(d)



wherein  $n$  is an integer from 0 to 8 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, or 8);  $p$  is an integer from 1 to 5 (e.g., 1, 2, 3, 4, or 5); and  $R_{13}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl;

10

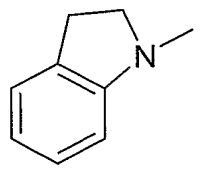
(e)



wherein  $q$  is an integer from 1 to 4 (e.g., 1, 2, 3, or 4); and  $R_{14}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl;

15

(f)

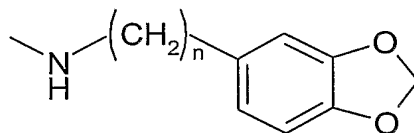


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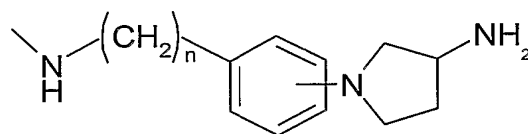
(g)



wherein n is an integer from 0 to 8 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, or 8); or

5

(h)

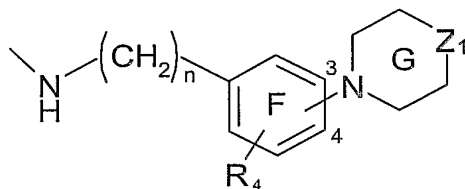


wherein n is an integer from 0 to 8 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, or 8).

In some embodiments, Y is  $\text{NR}_2\text{R}_3$  and  $\text{R}_1$  is nitro,  $\text{R}_2$  is H and  $\text{R}_3$  is aryl or substituted aryl. In other embodiments, Y is  $\text{NR}_2\text{R}_3$  and  $\text{R}_1$  is nitro, and  $\text{R}_2$  and  $\text{R}_3$  together form a ring structure with the nitrogen atom to which they are attached.

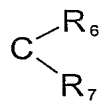
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In some embodiments, Y is:

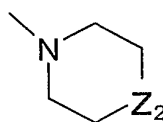


and n can be zero or one. Further, in some embodiments ring G is in the 3-position or 4-position of ring F. In some embodiments,  $\text{Z}_1$  is oxygen or sulfur. In other embodiments  $\text{Z}_1$  is  $\text{NR}_5$ . In still other embodiments,  $\text{Z}_1$  is

15

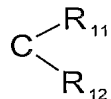


In some embodiments, Y is:



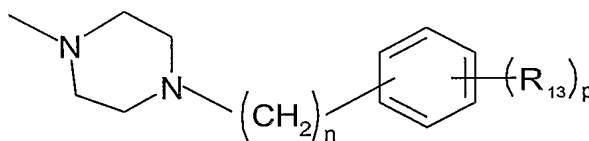
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and  $Z_2$  is  $NR_{10}$ , wherein  $R_{10}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl. In other embodiments,  $Z_2$  is



, wherein  $R_{11}$  and  $R_{12}$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl.

In some embodiments, Y is:



and n is zero or one.

In some embodiments of the novel compounds disclosed herein, Y can be anisidine, 3-halo-aniline, 3-anisidine, 4-anisidine, cyclohexylamine, adamantyl amine, furfuryl amine, 4-amino-benzonitrile, 4-methoxy-benzylamine, 2-chloro-benzylamine, 2,4-dimethoxy-benzylamine, 3,4-dimethoxy-benzylamine, 3,4,5-trimethoxy-benzylamine, 1-amino-1,2,3,4-tetrahydro-naphthalene, 1-amino-indane, phenethylamine, 4-ethoxy-phenethylamine, (S)-1-phenyl-ethylamine, (R)-1-phenyl-ethylamine, 3,4-dimethoxy-phenethylamine, 4-methoxy-benzylamine, 3-amino-phenol, 3-benzyloxy-aniline, N-methyl-aniline, N-methyl-4-anisidine, 2,3-dihydro-indole, 2-amino-pyridine, 3-amino-pyridine, 4-amino-pyridine, 3-amino-pyrazole, 2-amino-pyrazine, 2-amino methyl pyridine, 2-amino-4-methoxy-benzothiazole, 4-amino-6-methoxy-pyrimidine, 2-methoxy-benzylamine, or 2,3-dimethoxy-benzylamine. In particular embodiments, Y is 3-chloro-aniline, 3-fluoro-aniline, 3-anisidine, 4-methoxy-benzylamine, or 3,4-dimethoxy-benzylamine.

Particular compounds of the novel compounds disclosed herein include but are not limited to: 5-nitrofur-2-carboxylic acid (3-chloro-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-bromo-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-fluoro-phenyl)-amide; 5-nitrofur-2-carboxylic acid (4-methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-nitrofur-2-

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carboxylic acid adamantan-1-ylamide; 5-nitrofur-2-carboxylic acid  
phenylamide; 5-nitrofur-2-carboxylic acid (furan-2-ylmethyl)-amide; 5-  
nitrofur-2-carboxylic acid (4-cyano-phenyl)-amide; 5-nitrofur-2-carboxylic  
acid 4-methoxy-benzylamide; 5-nitrofur-2-carboxylic acid 2-  
5 chlorobenzylamide; 5-nitrofur-2-carboxylic acid 2,4-dimethoxybenzylamide;  
5-nitrofur-2-carboxylic acid 3,4-dimethoxybenzylamide; 5-nitrofur-2-  
carboxylic acid 3,4,5-trimethoxybenzylamide; 5-nitrofur-2-carboxylic acid  
(1,2,3,4-tetrahydro-naphthalen-1-yl)-amide; 5-nitrofur-2-carboxylic acid  
indan-1-ylamide; 5-nitrofur-2-carboxylic acid phenethyl-amide; 5-nitrofur-2-  
10 carboxylic acid [2-(4-methoxy-phenyl)-ethyl]-amide; 5-nitrofur-2-carboxylic  
acid (1-phenyl-ethyl)-amide; 5-nitrofur-2-carboxylic acid [2-(3,4-dimethoxy-  
phenyl)-ethyl]-amide; 5-nitrofur-2-carboxylic acid 4-methoxy-benzylamide; 5-  
nitrofur-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-sulfo-furan-2-  
carboxylic acid; 5-(3-methoxy-phenylcarbonyl)-furan-sulfonic acid; 5-  
15 nitrofur-2-carboxylic acid (3-hydroxy-phenyl)-amide; 5-phenylsulfanyl-furan-2-  
carboxylic acid (3-methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-  
benzyloxy-phenyl)-amide; 5-benzenesulfinyl-furan-2-carboxylic acid (3-  
methoxy-phenyl)-amide; 5-benzenesulfonyl-furan-2-carboxylic acid (3-methoxy-  
phenyl)-amide; 5-nitrofur-2-carboxylic acid pyrazin-2-ylamide; 5-nitrofur-2-  
20 carboxylic acid (pyridin-2-yl methyl)-amide; 5-nitrofur-2-carboxylic acid (4-  
methoxy-benzothiazol-2-yl)-amide; 5-nitrofur-2-carboxylic acid (6-methoxy-  
pyrimin-4-yl)-amide; 5-nitrofur-2-carboxylic acid 2-methoxy-benzylamide; 5-  
nitrofur-2-carboxylic acid 2,3-dimethoxy-benzylamide; 5-nitrofur-2-  
carboxylic acid 4-thiomorpholin-4-yl-benzylamide; 5-nitrofur-2-carboxylic acid  
25 4-(1-oxo-1,4-thiomorpholin-4-yl)-benzylamide; 5-nitrofur-2-carboxylic acid 4-  
(1,1-dioxo-1,4-thiomorpholin-4-yl)-benzylamide; 5-nitrofur-2-carboxylic acid  
4-(3-amino-pyrrolidin-1-yl)-benzylamide; 4-(4-[(5-nitrofur-2-carbonyl)-amino]-  
methyl)-phenyl)-piperazine-1-carboxylic acid tert-butyl ester; 5-nitrofur-2-  
carboxylic acid 4-piperazin-1-yl-benzylamide; 5-nitrofur-2-carboxylic acid 4-(4-  
30 cyclopropylmethyl-piperazin-1-yl)-benzylamide; 5-nitrofur-2-carboxylic acid 4-  
(4-benzyl-piperazin-1-yl)-3-fluoro-benzylamide; 5-nitrofur-2-carboxylic acid 3-

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fluoro-4-(4-methyl-piperazin-1-yl)-benzylamide; 5-nitrofur-2-carboxylic acid 3-fluoro-4-thiomorpholin-4-yl-benzylamide; 5-nitrofur-2-carboxylic acid 3-fluoro-4-morpholin-4-yl-benzylamide; 5-nitrofur-2-carboxylic acid 4-(4-benzyl-piperidin-1-yl)-3-fluoro-benzylamide; 4-(4-[[5-nitrofur-2-carbonyl]-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid ethyl ester; 4-(4-[[5-nitrofur-2-carbonyl]-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid propylamide; 4-(4-[[5-nitrofur-2-carbonyl]-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid isopropylamide; *N*-(4-methoxybenzyl)-5-nitrofur-2-carboxamide; (3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl)(5-nitrofur-2-yl)methanone; *N*-((benzo[*d*][1,3]dioxol-6-yl)methyl)-5-nitrofur-2-carboxamide; *N*-(2,4-dimethoxybenzyl)-5-nitrofur-2-carboxamide; *N*-(3,4-dimethoxyphenethyl)-5-nitrofur-2-carboxamide; *N*-(3,4-dimethoxybenzyl)-5-nitrofur-2-carboxamide; and *N*-(4-methoxyphenyl)-5-nitrofur-2-carboxamide.

#### B. Prodrugs

In representative embodiments, compounds disclosed herein are prodrugs. A prodrug means a compound that, upon administration to a recipient, is capable of providing (directly or indirectly) a compound of this invention or an inhibitorily active metabolite or residue thereof. Prodrugs can increase the bioavailability of the compounds of the presently disclosed subject matter when such compounds are administered to a subject (e.g., by allowing an orally administered compound to be more readily absorbed into the blood) or can enhance delivery of the parent compound to a biological compartment (e.g., the brain or lymphatic system) relative to a metabolite species, for example.

#### C. Pharmaceutically Acceptable Salts

Additionally, the active compounds can be administered as pharmaceutically acceptable salts. Such salts include the gluconate, lactate, acetate, tartarate, citrate, phosphate, borate, nitrate, sulfate, and hydrochloride salts. The salts of the compounds described herein can be prepared, in general, by reacting two equivalents of the base compound with the desired acid, in solution. After the reaction is complete, the salts are crystallized from

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solution by the addition of an appropriate amount of solvent in which the salt is insoluble.

### III. Methods of Utilizing Novel Compounds

5           The novel compounds disclosed herein have utility in killing or inhibiting the growth of microorganisms and in the treatment of subjects infected with microorganisms. As such, disclosed herein below are methods of killing or inhibiting the growth a microorganism comprising contacting the microorganism with an effective amount of a novel compound disclosed herein. Also disclosed  
10       herein below are methods of treating a microbial infection in a subject comprising administering to the subject a therapeutically effective amount of a novel compound disclosed herein.

          Microorganisms killed or growth-inhibited and microbial infections treated by the novel compounds and methods disclosed herein include a variety of  
15       microbes, including fungi, algae, protozoa, bacteria, and viruses. Exemplary microorganisms killed or growth-inhibited and microbial infections that can be treated by the methods of the presently disclosed subject matter include, but are not limited to, infections caused by bacteria, specifically members of the genus *Mycobacterium*. As a non-limiting example, the members can include  
20       *Mycobacterium tuberculosis*, which can cause the disease tuberculosis, in all its forms, in animal subjects.

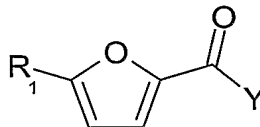
          The methods disclosed herein are useful for treating these conditions in that they inhibit the onset, growth, or spread of the condition, cause regression of the condition, cure the condition, or otherwise improve the general well-being  
25       of a subject afflicted with, or at risk of contracting the condition.

          Methods of killing or inhibiting the growth of a microorganism or treating a microbial infection comprise contacting the microorganism with, or administering to a subject in need of treatment, respectively, an active compound as described herein. These active compounds, as set forth above,  
30       include the compounds, their corresponding prodrugs, and pharmaceutically acceptable salts of the compounds and prodrugs.



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With regard to the presently described method embodiments, representative compounds can have a structure as follows:

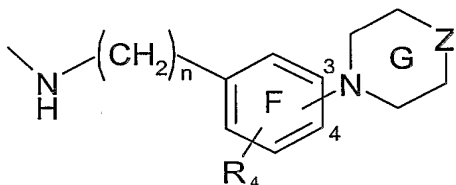


wherein  $R_1$  is selected from the group consisting of halo, nitro, alkyl ester, phenylsulfanyl, phenylsulfinyl, phenylsulfonyl and sulfonic acid; and Y is a substituted amine.

In some embodiments of the novel compounds, Y can be:

(a)  $-NR_2R_3$ , wherein  $R_2$  and  $R_3$  are each independently selected from the group consisting of H, alkyl, aryl, substituted alkyl, and substituted aryl, or  $R_2$  and  $R_3$  together form a ring structure with the nitrogen atom to which they are attached;

(b)

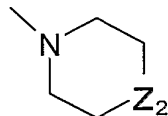


wherein  $n$  is an integer from 0 to 8;  $R_4$  is selected from the group consisting of hydrogen, halo, alkyl, substituted alkyl, and alkoxy; and  $Z_1$  is selected from the group consisting of oxygen, sulfur, sulfoxide,

sulfone,  $NR_5$ , and  $C(R_6)(R_7)$ ; wherein  $R_5$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkoxy, carbonyl, aryl, substituted aryl, and  $-(C=O)-NR_8R_9$ , wherein  $R_6$  and  $R_7$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl and  $R_8$  and  $R_9$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl;

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(c)

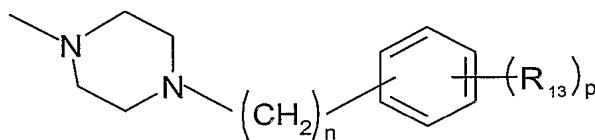


wherein  $Z_2$  is selected from the group consisting of oxygen,  $NR_{10}$ ,

5

and  $\begin{array}{c} R_{11} \\ \diagup \\ C \\ \diagdown \\ R_{12} \end{array}$ ;  $R_{10}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl; and  $R_{11}$  and  $R_{12}$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl;

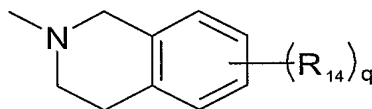
(d)



10

wherein  $n$  is an integer from 0 to 8;  $p$  is an integer from 1 to 5; and  $R_{13}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl;

(e)

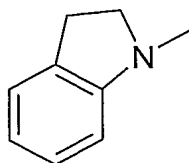


15

wherein  $q$  is an integer from 1 to 4; and  $R_{14}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl;

20

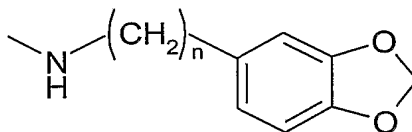
(f)



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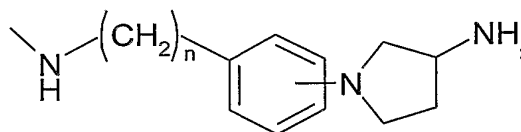
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(g)



wherein n is an integer from 0 to 8; or

(h)



wherein n is an integer from 0 to 8.

In some embodiments of the novel compounds disclosed herein, Y can be anisidine, 3-halo-aniline, 3-anisidine, 4-anisidine, cyclohexylamine, adamantyl amine, furfuryl amine, 4-amino-benzonitrile, 4-methoxy-benzylamine, 2-chloro-benzylamine, 2,4-dimethoxy-benzylamine, 3,4-dimethoxy-benzylamine, 3,4,5-trimethoxy-benzylamine, 1-amino-1,2,3,4-tetrahydro-naphthalene, 1-amino-indane, phenethylamine, 4-ethoxy-phenethylamine, (S)-1-phenyl-ethylamine, (R)-1-phenyl-ethylamine, 3,4-dimethoxy-phenethylamine, 4-methoxy-benzylamine, 3-amino-phenol, 3-benzyloxy-aniline, N-methyl-aniline, N-methyl-4-anisidine, 2,3-dihydro-indole, 2-amino-pyridine, 3-amino-pyridine, 4-amino-pyridine, 3-amino-pyrazole, 2-amino-pyrazine, 2-amino methyl pyridine, 2-amino-4-methoxy-benzothiazole, 4-amino-6-methoxy-pyrimidine, 2-methoxy-benzylamine, or 2,3-dimethoxy-benzylamine. In particular embodiments, Y is 3-chloro-aniline, 3-fluoro-aniline, 3-anisidine, 4-methoxy-benzylamine, or 3,4-dimethoxy-benzylamine.

Particular compounds of the novel compounds disclosed herein include but are not limited to: 5-nitrofur-2-carboxylic acid (3-chloro-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-bromo-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-fluoro-phenyl)-amide; 5-nitrofur-2-carboxylic acid (4-methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid adamantan-1-ylamide; 5-nitrofur-2-carboxylic acid phenylamide; 5-nitrofur-2-carboxylic acid (furan-2-ylmethyl)-amide; 5-

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nitrofuran-2-carboxylic acid (4-cyano-phenyl)-amide; 5-nitrofuran-2-carboxylic acid 4-methoxy-benzylamide; 5-nitrofuran-2-carboxylic acid 2-chlorobenzylamide; 5-nitrofuran-2-carboxylic acid 2,4-dimethoxybenzylamide; 5-nitrofuran-2-carboxylic acid 3,4-dimethoxybenzylamide; 5-nitrofuran-2-carboxylic acid 3,4,5-trimethoxybenzylamide; 5-nitrofuran-2-carboxylic acid (1,2,3,4-tetrahydro-naphthalen-1-yl)-amide; 5-nitrofuran-2-carboxylic acid indan-1-ylamide; 5-nitrofuran-2-carboxylic acid phenethyl-amide; 5-nitrofuran-2-carboxylic acid [2-(4-methoxy-phenyl)-ethyl]-amide; 5-nitrofuran-2-carboxylic acid (1-phenyl-ethyl)-amide; 5-nitrofuran-2-carboxylic acid [2-(3,4-dimethoxy-phenyl)-ethyl]-amide; 5-nitrofuran-2-carboxylic acid 4-methoxy-benzylamide; 5-nitrofuran-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-sulfo-furan-2-carboxylic acid; 5-(3-methoxy-phenylcarbamoyl)-furan-sulfonic acid; 5-nitrofuran-2-carboxylic acid (3-hydroxy-phenyl)-amide; 5-phenylsulfanyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-nitrofuran-2-carboxylic acid (3-benzyloxy-phenyl)-amide; 5-benzenesulfinyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-benzenesulfonyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-nitrofuran-2-carboxylic acid pyrazin-2-ylamide; 5-nitrofuran-2-carboxylic acid (pyridin-2-yl methyl)-amide; 5-nitrofuran-2-carboxylic acid (4-methoxy-benzothiazol-2-yl)-amide; 5-nitrofuran-2-carboxylic acid (6-methoxy-pyrimin-4-yl)-amide; 5-nitrofuran-2-carboxylic acid 2-methoxy-benzylamide; 5-nitrofuran-2-carboxylic acid 2,3-dimethoxy-benzylamide; 5-nitrofuran-2-carboxylic acid 4-thiomorpholin-4-yl-benzylamide; 5-nitrofuran-2-carboxylic acid 4-(1-oxo-1,4-thiomorpholin-4-yl)-benzylamide; 5-nitrofuran-2-carboxylic acid 4-(1,1-dioxo-1,4-thiomorpholin-4-yl)-benzylamide; 5-nitrofuran-2-carboxylic acid 4-(3-amino-pyrrolidin-1-yl)-benzylamide; 4-(4-[(5-nitrofuran-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid tert-butyl ester; 5-nitrofuran-2-carboxylic acid 4-piperazin-1-yl-benzylamide; 5-nitrofuran-2-carboxylic acid 4-(4-cyclopropylmethyl-piperazin-1-yl)-benzylamide; 5-nitrofuran-2-carboxylic acid 4-(4-benzyl-piperazin-1-yl)-3-fluoro-benzylamide; 5-nitrofuran-2-carboxylic acid 3-fluoro-4-(4-methyl-piperazin-1-yl)-benzylamide; 5-nitrofuran-2-carboxylic acid 3-fluoro-4-thiomorpholin-4-yl-benzylamide; 5-nitrofuran-2-carboxylic acid 3-fluoro-

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4-morpholin-4-yl-benzylamide; 5-nitrofuranyl-2-carboxylic acid 4-(4-benzyl-  
piperidin-1-yl)-3-fluoro-benzylamide; 4-(4-[(5-nitrofuranyl-2-carbonyl)-amino]-  
methyl)-phenyl)-piperazine-1-carboxylic acid ethyl ester; 4-(4-[(5-nitrofuranyl-2-  
carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid propylamide; 4-  
5 (4-[(5-nitrofuranyl-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic  
acid isopropylamide; *N*-(4-methoxybenzyl)-5-nitrofuranyl-2-carboxamide; (3,4-  
dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl)(5-nitrofuranyl-2-yl)methanone; *N*-  
((benzo[*d*][1,3]dioxol-6-yl)methyl)-5-nitrofuranyl-2-carboxamide; *N*-(2,4-  
dimethoxybenzyl)-5-nitrofuranyl-2-carboxamide; *N*-(3,4-dimethoxyphenethyl)-5-  
10 nitrofuranyl-2-carboxamide; *N*-(3,4-dimethoxybenzyl)-5-nitrofuranyl-2-  
carboxamide; and *N*-(4-methoxyphenyl)-5-nitrofuranyl-2-carboxamide.

An effective amount of any specific active compound, the use of which is  
in the scope of embodiments described herein, will vary somewhat from  
compound to compound, use to use (for example, specifically killing or  
15 inhibiting the growth of a microorganism or treating a microbial infection in a  
subject), and subject to subject when utilizing methods of treating subjects, and  
will depend upon the condition of the patient and the route of delivery.

As a general proposition, an effective amount is from about 1 to about  
1000  $\mu\text{m/mL}$  of the compound. In some embodiments, the effective amount is  
20 from about 10 to 500  $\mu\text{m/mL}$ . In other embodiments, the effective amount is  
from about 50 to 250  $\mu\text{m/mL}$ . In some particular embodiments, the effective  
amount is from about 100 to 200  $\mu\text{m/mL}$ .

The subject treated in the presently disclosed subject matter in its many  
embodiments is desirably a human subject, although it is to be understood the  
25 methods described herein are effective with respect to all vertebrate species,  
which are intended to be included in the term "subject". The methods  
described herein are particularly useful in the treatment and/or prevention of  
microbial infections in warm-blooded vertebrates. Thus, the methods can be  
used as treatment for mammals and birds.

30 More particularly, provided is the treatment of mammals such as  
humans, as well as those mammals of importance due to being endangered

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(such as Siberian tigers), of economical importance (animals raised on farms for consumption by humans) and/or social importance (animals kept as pets or in zoos) to humans, for instance, carnivores other than humans (such as cats and dogs), swine (pigs, hogs, and wild boars), ruminants (such as cattle, oxen, sheep, giraffes, deer, goats, bison, and camels), and horses. Also provided is the treatment of birds, including the treatment of those kinds of birds that are endangered, kept in zoos, as well as fowl, and more particularly domesticated fowl, i.e., poultry, such as turkeys, chickens, ducks, geese, guinea fowl, and the like, as they are also of economic importance to humans. Thus, embodiments of the methods described herein include the treatment of livestock, including, but not limited to, domesticated swine (pigs and hogs), ruminants, horses, poultry, and the like.

#### IV. Pharmaceutical Formulations

The novel compounds disclosed herein, the pharmaceutically acceptable salts thereof, prodrugs corresponding to the novel compounds disclosed herein, and the pharmaceutically acceptable salts thereof, are all referred to herein as "active compounds." Pharmaceutical formulations comprising the aforementioned active compounds are also provided herein. These pharmaceutical formulations comprise active compounds as described herein, in a pharmaceutically acceptable carrier. Pharmaceutical formulations can be prepared for oral, intravenous, or aerosol administration as discussed in greater detail below. Also, the present invention provides such active compounds that have been lyophilized and that can be reconstituted to form pharmaceutically acceptable formulations for administration, as by intravenous or intramuscular injection.

The therapeutically effective dosage of any specific active compound, the use of which is in the scope of embodiments described herein, will vary somewhat from compound to compound, and patient to patient, and will depend upon the condition of the patient and the route of delivery. As a general proposition, a dosage from about 1 to about 1000  $\mu\text{m/mL}$  of the

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compound within the formulation is considered an effective dosage. In some embodiments, the effective amount is from about 10 to 500  $\mu\text{m/mL}$ . In other embodiments, the effective amount is from about 50 to 250  $\mu\text{m/mL}$ . In some particular embodiments, the effective amount is from about 100 to 200  $\mu\text{m/mL}$ .

5           In accordance with the present methods, pharmaceutically active compounds as described herein can be administered orally as a solid or as a liquid, or can be administered intramuscularly or intravenously as a solution, suspension, or emulsion. Alternatively, the compounds or salts can also be administered by inhalation, intravenously or intramuscularly as a liposomal  
10           suspension. When administered through inhalation the active compound or salt should be in the form of a plurality of solid particles or droplets having a particle size from about 0.5 to about 5 microns, and preferably from about 1 to about 2 microns.

          Pharmaceutical formulations suitable for intravenous or intramuscular  
15           injection are further embodiments provided herein. The pharmaceutical formulations comprise a compound of Formula I described herein, a prodrug as described herein, or a pharmaceutically acceptable salt thereof, in any pharmaceutically acceptable carrier. If a solution is desired, water is the carrier of choice with respect to water-soluble compounds or salts. With respect to the  
20           water-soluble compounds or salts, an organic vehicle, such as glycerol, propylene glycol, polyethylene glycol, or mixtures thereof, can be suitable. In the latter instance, the organic vehicle can contain a substantial amount of water. The solution in either instance can then be sterilized in a suitable manner known to those in the art, and typically by filtration through a 0.22-  
25           micron filter. Subsequent to sterilization, the solution can be dispensed into appropriate receptacles, such as depyrogenated glass vials. Of course, the dispensing is preferably done by an aseptic method. Sterilized closures can then be placed on the vials and, if desired, the vial contents can be lyophilized.

          In addition to the novel compounds disclosed herein, or their salts or  
30           prodrugs, the pharmaceutical formulations can contain other additives, such as pH-adjusting additives. In particular, useful pH-adjusting agents include acids,

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such as hydrochloric acid, bases or buffers, such as sodium lactate, sodium acetate, sodium phosphate, sodium citrate, sodium borate, or sodium gluconate. Further, the formulations can contain anti-microbial preservatives. Useful anti-microbial preservatives include methylparaben, propylparaben, and benzyl alcohol. The anti-microbial preservative is typically employed when the formulation is placed in a vial designed for multi-dose use. The pharmaceutical formulations described herein can be lyophilized using techniques well known in the art.

In yet another aspect of the subject matter described herein, there is provided an injectable, stable, sterile formulation comprising a novel compound disclosed herein, or a salt thereof, in a unit dosage form in a sealed container. The compound or salt is provided in the form of a lyophilizate, which is capable of being reconstituted with a suitable pharmaceutically acceptable carrier to form a liquid formulation suitable for injection thereof into a subject. The unit dosage form typically comprises from about 10 mg to about 10 grams of the compound salt. When the compound or salt is substantially water-insoluble, a sufficient amount of emulsifying agent, which is physiologically acceptable, can be employed in sufficient quantity to emulsify the compound or salt in an aqueous carrier. One such useful emulsifying agent is phosphatidyl choline.

Other pharmaceutical formulations can be prepared from the water-insoluble compounds disclosed herein, or salts thereof, such as aqueous base emulsions. In such an instance, the formulation will contain a sufficient amount of pharmaceutically acceptable emulsifying agent to emulsify the desired amount of the compound or salt thereof. Particularly useful emulsifying agents include phosphatidyl cholines, and lecithin.

Additional embodiments provided herein include liposomal formulations of the active compounds disclosed herein. The technology for forming liposomal suspensions is well known in the art. When the compound is an aqueous-soluble salt, using conventional liposome technology, the same can be incorporated into lipid vesicles. In such an instance, due to the water solubility of the active compound, the active compound will be substantially



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entrained within the hydrophilic center or core of the liposomes. The lipid layer employed can be of any conventional composition and can either contain cholesterol or can be cholesterol-free. When the active compound of interest is water-insoluble, again employing conventional liposome formation technology, the salt can be substantially entrained within the hydrophobic lipid bilayer that forms the structure of the liposome. In either instance, the liposomes that are produced can be reduced in size, as through the use of standard sonication and homogenization techniques.

The liposomal formulations containing the active compounds disclosed herein can be lyophilized to produce a lyophilizate, which can be reconstituted with a pharmaceutically acceptable carrier, such as water, to regenerate a liposomal suspension.

Pharmaceutical formulations are also provided which are suitable for administration as an aerosol, by inhalation. These formulations comprise a solution or suspension of a desired compound described herein or a salt thereof, or a plurality of solid particles of the compound or salt. The desired formulation can be placed in a small chamber and nebulized. Nebulization can be accomplished by compressed air or by ultrasonic energy to form a plurality of liquid droplets or solid particles comprising the compounds or salts. The liquid droplets or solid particles should have a particle size in the range of about 0.5 to about 10 microns, more preferably from about 0.5 to about 5 microns. The solid particles can be obtained by processing the solid compound or a salt thereof, in any appropriate manner known in the art, such as by micronization. Most preferably, the size of the solid particles or droplets will be from about 1 to about 2 microns. In this respect, commercial nebulizers are available to achieve this purpose. The compounds can be administered via an aerosol suspension of respirable particles in a manner set forth in U.S. Patent No. 5,628,984, the disclosure of which is incorporated herein by reference in its entirety.

When the pharmaceutical formulation suitable for administration as an aerosol is in the form of a liquid, the formulation will comprise a water-soluble

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active compound in a carrier that comprises water. A surfactant can be present, which lowers the surface tension of the formulation sufficiently to result in the formation of droplets within the desired size range when subjected to nebulization.

5           As indicated, both water-soluble and water-insoluble active compounds are provided. As used in the present specification, the term "water-soluble" is meant to define any composition that is soluble in water in an amount of about 50 mg/mL, or greater. Also, as used in the present specification, the term "water-insoluble" is meant to define any composition that has solubility in water  
10 of less than about 20 mg/mL. For certain applications, water-soluble compounds or salts can be desirable whereas for other applications water-insoluble compounds or salts likewise can be desirable.

#### EXAMPLES

15           The following Examples have been included to illustrate modes of the presently disclosed subject matter. Certain aspects of the following Examples are described in terms of techniques and procedures found or contemplated to work well in the practice of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can  
20 appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter.

##### Example 1

25           Galactofuranose is an essential component of the mycobacterial cell wall and not found in humana, UDP-galactofuranose is biosynthesized from UDP-galactopyranose using the enzyme UDP-galactose mutase (Glf). Disclosed herein is a microtitre plate based screen of Glf used to discover novel inhibitors as potential new anti-tuberculosis agents. In the course of using the screen  
30 nitrofuranylamide **1** was discovered to be an inhibitor of Glf with an IC<sub>50</sub> of 7  $\mu$ g/mL. Noticeably, this compound had good activity against whole cells with an

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MIC of 1.6  $\mu\text{g/mL}$ . Example 1 describes efforts at developing the structure activity relationship of compound **1** with respect to Glf inhibition and anti-tuberculosis activity, as well as deriving other even more effective compounds having anti-tuberculosis activity.

5

#### Methods and Materials

All the anhydrous solvents and starting materials were purchased from Aldrich Chemical Company (Milwaukee, Wisconsin, U.S.A.). All reagent grade solvents used for chromatography were purchased from Fisher Scientific (Suwanee, Georgia, U.S.A.) and FLASH<sup>TM</sup> column chromatography silica  
10 cartridges were obtained from Biotage Inc. (Lake Forest, Virginia, U.S.A.). The reactions were monitored by thin layer chromatography (TLC) on pre-coated Merck 60 F<sub>254</sub> silica gel plates and visualized using UV light (254 nm). Biotage FLASH 25+<sup>TM</sup> column chromatography system was used to purify mixtures. All <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker ARX-300 (300 and 75  
15 MHz for <sup>1</sup>H and <sup>13</sup>C NMR, respectively; Billerica, Massachusetts, U.S.A.) or Varian INOVA-500<sup>TM</sup> (500 and 125 MHz for <sup>1</sup>H and <sup>13</sup>C NMR, respectively; Palo Alto, California, U.S.A.) spectrometer. Chemical shifts are reported in ppm ( $\delta$ ) relative to residual solvent peak or internal standard (tetramethylsilane) and coupling constants (*J*) are reported in hertz (Hz). Mass spectra were recorded  
20 on a Bruker ESQUIRE LCMS<sup>TM</sup> using ESI. Purity of the final products was confirmed before testing by analytical HPLC using a Alltech (Deerfield, Illinois, U.S.A.) platinum C-18 reverse phase column (4.5 x 150mm) and a H<sub>2</sub>O (0.1% TFA) to acetonitrile 0-100% linear gradient at a flow rate of 1.0 mL min<sup>-1</sup> and UV detection at 254nm.

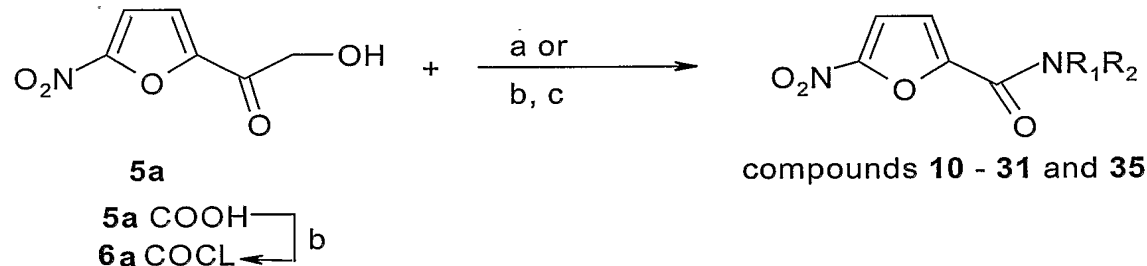
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#### Scheme 1

Referring now to Scheme 1, the synthesis of nitrofuranyl amides **10-31** and **35** were prepared by reacting the corresponding amines with 5-nitro-2-furan carboxylic acid **5a** in presence of EDCI (Scheme 1) according to the parallel synthesis protocol of Boger. This route was later replaced when  
30 coupling less reactive heteroaromatic amines by using acid chloride chemistry

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**6a** to form amides **41-53**. This route has proved to be more cost effective and scalable.



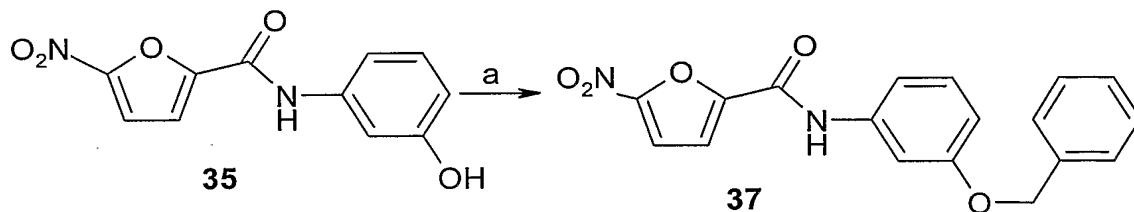
5 Reagents: a) EDCl, DMAP, DMF, room temp., 14 hr.; b) (COCl)<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, Cat. DMF; c) HNR<sub>1</sub>R<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, NEt<sub>3</sub>, 4 hr., room temp., or 47 °C (or) HNR<sub>1</sub>R<sub>2</sub>, DMF, Py, 60 °C, 4 hr.

### Scheme 2

10 Referring now to Scheme 2, nitrofuranyl amide **35** was further elaborated into amide **37** by benzylating the phenolic hydroxyl group using standard benzylation conditions.

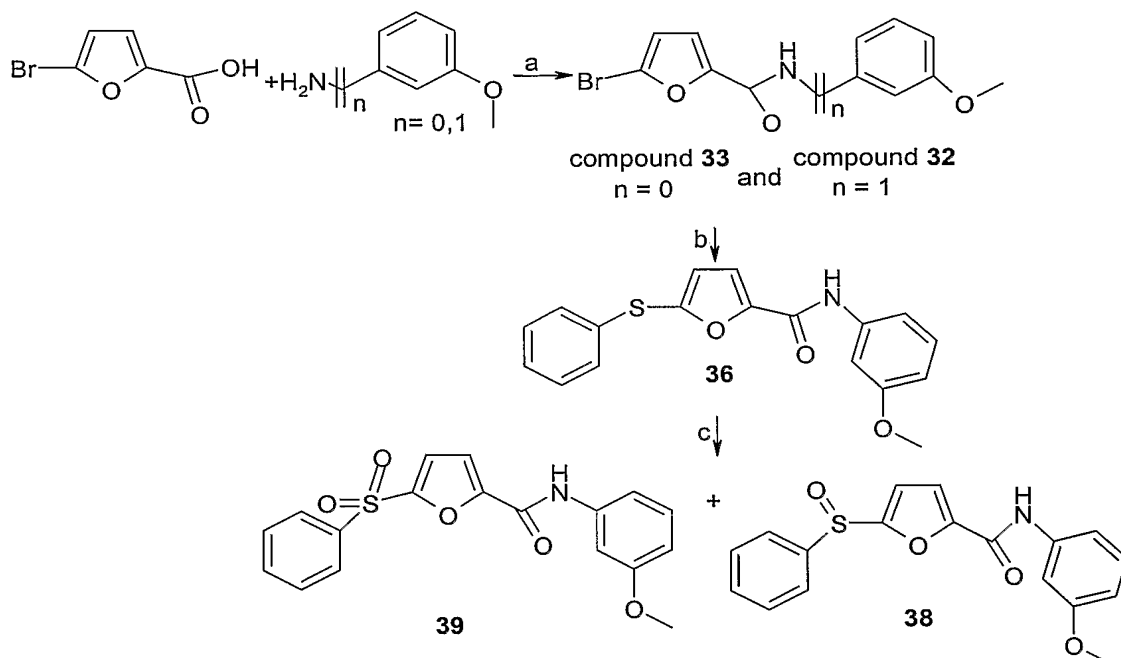
15 In order to evaluate the importance of the nitro functionality on 5-position of furan ring other furanyl amides were synthesized. Accordingly, the 5-bromofuranyl amides **32**, and **33** were synthesized by reacting 5-bromo furan carboxylic acid with corresponding amines in presence of EDCl (Scheme 2). The 5-bromo substitution on furan ring was subsequently used to effect further transformations at the 5-position. Bromofuranyl amide **33** was converted to thioether **36** by a nucleophilic substitution of the bromine on furan ring with thiophenol. The thioether **36** upon oxidation provided sulfoxide and sulfone amides **38** and **39** (Scheme 2).

20



Reagents: a) BnBr, K<sub>2</sub>CO<sub>3</sub>, THF, room temp., 12 hr.

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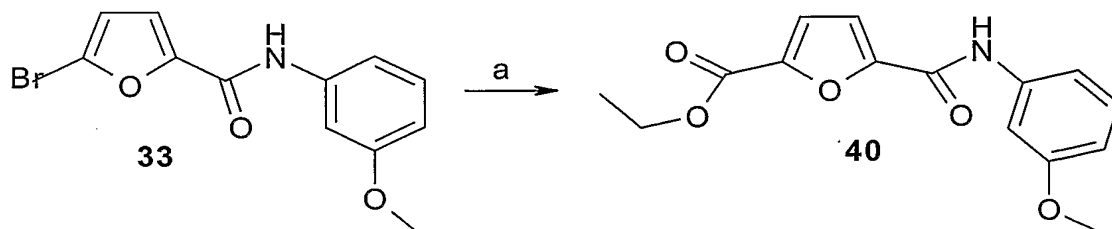
Reagents: a) EDCI, DMAP, DMF, room temp., 14 hr.; b) PhSH, NaH, 150 °C, 12 hr., c) m-CPBA, CH<sub>2</sub>Cl<sub>2</sub>, NaCO<sub>3</sub>, 0 °C, 3 hr.

5

### Scheme 3

Referring now to Scheme 3, furanylamide bromide **33** was also converted to furanylamide ester **40** using standard Grignard chemistry (Scheme 3). Treatment of the bromide with ethyl magnesium bromide followed by reaction of the intermediate with ethylchloroformate afforded the target ester **40**.

10



Reagents: a) ClCOOEt, EtMgBr, EtBr, dry THF, 0 °C – room temp.

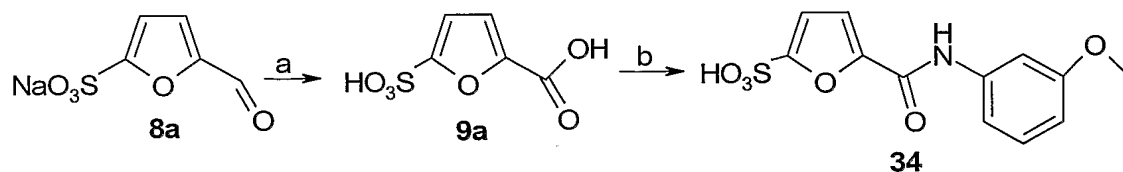
### Scheme 4

15

Referring now to Scheme 4, the sulfonate analog **34** was synthesized from commercially available aldehyde **8a**, which was oxidized to acid **9a** with

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silver nitrate and coupled to *m*-anisidine using the standard EDCI mediated coupling protocol to yield amide **34** (Scheme 4).



5 Reagents: a) AgNO<sub>3</sub>, aq. NaOH, room temp., 0.5 hr; b) EDCI, DMAP, DMF, room temp.

**General procedure for preparation of amides 10-30, 32, 33 and 35.**

5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol, 1 equiv) and amine (1.9 mmol, 1 equiv) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol, 2 equiv) followed by DMAP (582 mg, 4.7 mmol, 2.5 equiv) and the resulting solution was stirred for 14 hr. at 25 °C. The reaction mixture was poured into EtOAc (75 mL) and washed with 10% aqueous HCl (2 x 50 mL) and washed with 10% aqueous NaHCO<sub>3</sub> (3 x 50 mL). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated followed by flash-column purification with Pet. Ether and EtOAc system provided corresponding amides (yields generally vary from 69% to 92%).

**5-Nitro-furan-2-carboxylic acid (3-chloro-phenyl)-amide (10).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and *m*-chloro aniline (202  $\mu$ L, 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 432 mg product (85% yield). TLC: R<sub>f</sub> 0.82 (1:hexane:ethyl acetate); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$ 7.23 (1H, ddd, J = 7.8 Hz, 2.0 Hz, 1.0 Hz), 7.35 (1H, t, J = 7.8 Hz), 7.44 (2H, q, J = 9.0 Hz, 3.8 Hz), 7.54 (1H, ddd, J = 7.8 Hz, 2.0 Hz, 1.0 Hz), 7.84 (1H, t, J = 2.0 Hz), 8.27-8.33 (1H, bs); <sup>13</sup>C NMR (300 MHz, CDCl<sub>3</sub>): 112.09, 116.58, 117.85, 116.58, 120.02, 125.10, 129.70, 134.45, 136.93, 146.88, 153.45; EI-Mass: 265 (M<sup>+</sup>-1).

**5-Nitro-furan-2-carboxylic acid (3-bromo-phenyl)-amide (11).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and *m*-bromo aniline (306  $\mu$ L,

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1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 469 mg product (79% yield). TLC:  $R_f$  0.82 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$ 7.28 (1H, t,  $J$  = 7.7 Hz), 7.36 (1H, t,  $J$  = 1.4 Hz), 7.43 (2H, q,  $T$  = 9.6 Hz, 3.8 Hz), 7.6 (1H, ddd,  $J$  = 7.7 Hz, 2.1 Hz, 1.2 Hz), 7.98 (1H, t,  $J$  2.1 Hz), 8.23-8.3 (1H, bs);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 112.05, 116.54, 118.39, 122.87, 127.95, 129.95, 137.20, 140.72, 146.94, 153.50, 158.97; EI-Mass: 310.8 ( $\text{M}^+ - 1$ ).

**5-Nitro-furan-2-carboxylic acid (3-fluoro-phenyl)-amide (12).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and m-fluoro aniline (184  $\mu\text{L}$ , 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 429 mg product (89% yield).  $R_f$  0.82 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$ 6.9-6.98 (1H, m), 7.33-7.4 (2Hs, m), 7.44 (2Hs, q,  $J$  = 8.8 Hz, 3.8 Hz), 7.64-7.7 (1H, m), 8.3-8.4 (1H, bs);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 164.07, 160.81, 153.44, 146.91, 137.35, 129.93, 116.55, 115.16, 112.09, 107.64; EI-Mass: 248.8 ( $\text{M}^+ - 1$ ).

**5-Nitro-furan-2-carboxylic acid (3-methoxy-phenyl)-amide (13).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and m-anisidine (214  $\mu\text{L}$ , 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 450 mg of product (90% yield). TLC:  $R_f$  0.75 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$ 6.79 (1H, ddd,  $J$  = 8.4 Hz, 2.8 Hz, 1.2 Hz), 7.19 (1H, ddd,  $J$  = 8.4 Hz, 2.16 Hz, 0.7 Hz), 7.32 (1H, t,  $J$  = 8.4 Hz), 7.39-7.45 (2Hs, m), 8.22-8.28 (1H, bs);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 54.83, 105.65, 110.83, 112.06, 112.13, 116.20, 129.39, 137.06, 147.35, 153.48, 159.71; EI-Mass: 260.8. ( $\text{M}^+ - 1$ ).

**5-Nitro-furan-2-carboxylic acid (4-methoxy-phenyl)-amide (14).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and p-anisidine (234 mg, 1.9

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mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 425 mg of product (85% yield). TLC:  $R_f$  0.7 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$ 6.95 (1H, d,  $J$  = 8.9 Hz), 7.38 (1H, d,  $J$  = 3.9 Hz), 7.44 (1H, d,  $J$  = 3.9 Hz), 7.6 (1H, d,  $J$  = 8.9 Hz), 8.15-8.21 (1H, bs);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 112.11, 113.88, 115.94, 121.67, 128.81, 147.55, 153.29, 156.78, 157.1; EI-Mass: 260.9 ( $\text{M}^+ - 1$ ).

**5-Nitro-furan-2-carboxylic acid cyclohexylamide (15).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and cyclohexylamine (217  $\mu\text{L}$ , 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 322 mg of product (71% yield). TLC:  $R_f$  0.72 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$ 1.15-1.5 (6Hs, m), 1.82 (2Hs, dt,  $J$  = 9.7 Hz, 2.9 Hz), 2.03 (2 Hs, dd,  $J$  = 12.3 Hz, 2.6), 3.97 (1H, m), 6.42 (1H, bd,  $J$  = 6.2 Hz), 7.26 (1H, d,  $J$  = 3.8 Hz), 7.38 (1H, d,  $J$  = 3.8 Hz);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 23.25, 32.44, 33.06, 50.96, 111.97, 115.10, 147.85, 115.28; EI-Mass: 261.1 ( $\text{M}^+ + 23$ ).

**5-Nitro-furan-2-carboxylic acid adamantan-1-ylamide (16).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and adamantylamine (288 mg, 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 382 mg of product (69% yield). TLC:  $R_f$  0.72 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$ 1.70 (6Hs, s), 2.13 (9Hs, s), 6.18-6.25 (1H, bs), 7.2 (1H, d,  $J$  = 3.7 Hz), 7.35 (1H, d,  $J$  = 3.7 Hz);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 28.88, 35.65, 40.95, 52.62, 111.97, 114.78, 148.38, 154.58; EI-Mass: 288.9 ( $\text{M}^+ - 1$ ).

**5-Nitro-furan-2-carboxylic acid phenylamide (17).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and aniline (152  $\mu\text{L}$ , 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked



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up as explained in general procedure to afford 376 mg of product (85% yield). TLC:  $R_f$  0.75 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.24 (1H, tt,  $J = 7.9$  Hz, 0.8 Hz), 7.39-7.48 (4Hs, m), 7.7 (2H, dd,  $J = 8.4$  Hz, 0.8 Hz), 8.22-8.28 (1K, bs);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 112.10, 116.21, 119.90, 125.03, 128.73, 135.83, 147.34, 153.42; EI-Mass: 230.8 ( $\text{M}^+ - 1$ ).

**5-Nitro-furan-2-carboxylic acid (furan-2-ylmethyl)-amide (18).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and 2-aminomethyl furan (92  $\mu\text{L}$ , 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 383 mg of product (85% yield). TLC:  $R_f$  0.68 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  4.67 (2Hs, d,  $J = 5.1$  Hz, 3.0 Hz, 2.0 Hz, 0.4 Hz), 6.33-6.4 (2H, m), 6.86-6.94 (1H, bs), 7.32 (1H, d,  $J = 3.9$  Hz), 7.38 (1H, d,  $J = 3.9$  Hz), 7.42 (1H, dd,  $J = 2.06$  Hz, 0.4 Hz);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 35.82, 107.83, 110.04, 111.81, 115.69, 142.14, 147.23, 149.36, 155.48, 160.59; EI-Mass: 234.8 ( $\text{M}^+ - 1$ );

**5-Nitro-furan-2-carboxylic acid (4-cyano-phenyl)-amide (19).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and 4-amino benzonitrile (225 mg, 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 441 mg of product (90% yield). TLC:  $R_f$  0.62 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.52 (1H, d,  $J = 3.9$  Hz), 7.61 (1H, d,  $J = 3.9$  Hz), 7.76 (1H, d,  $J = 8.9$  Hz), 7.98 (1H, d,  $J = 8.9$  Hz);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 106.23, 113.29, 117.24, 118.74, 120.51, 133.13, 142.09, 147.17, 151.84, 154.89; EI-Mass: 255.8 ( $\text{M}^+ - 1$ ).

**5-Nitro-furan-2-carboxylic acid 4-methoxy-benzylamide (20).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and 4-methoxy benzylamine (248  $\mu\text{L}$ , 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to

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afford 448 mg of product (85% yield). TLC:  $R_f$  0.55 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$ 3.83 (3Hs, s), 4.58 (2Hs, d,  $J$  = 5.8 Hz), 6.82-6.92 (1H, bs), 6.92 (2Hs, d,  $J$  = 8 Hz), 7.27-7.32 (3Hs, m), 7.38 (1H, d,  $J$  = 3.5 Hz);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 42.57, 54.78, 111.87, 113.73, 115.49, 128.56, 128.92, 147.51, 155.56, 158.85; EI-Mass: 275.6 ( $\text{M}^+ - 1$ ).

**5-Nitro-furan-2-carboxylic acid 2-chloro-benzylamide (21).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and 2-chlorobenzylamine (230  $\mu\text{L}$ , 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 454 mg product (85% yield). TLC:  $R_f$  0.72 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$ 4.75 (2Hs, d,  $J$  = 6.2 Hz), 7.68-7.09 (1H, bs), 7.27-7.32 (3Hs, m), 7.38 (1H, d,  $J$  = 3.9 Hz), 7.41-7.49 (2Hs, m);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 40.99, 111.82, 115.64, 126.69, 128.91, 129.21, 129.87, 133.27, 133.90, 147.28, 155.67; EI-Mass: 278.8 ( $\text{M}^+ - 1$ ).

**5-Nitro-furan-2-carboxylic acid 2,4-dimethoxy-benzylamide (22).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and 2,4-dimethoxybenzylamine (286  $\mu\text{L}$ , 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 508 mg of product (87% yield). TLC:  $R_f$  0.50 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$ 3.83 (3Hs, s), 3.91 (3Hs, s), 4.57 (2Hs, d,  $J$  = 5.8 Hz), 6.45-6.53 (2Hs, m), 7.02-7.12 (1H, bs), 7.24 (1H, s), 7.27 (1H, d,  $J$  = 2.5 Hz), 7.36 (1H, d,  $J$  = 2.5 Hz);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 38.55, 54.88, 54.91, 98.19, 103.65, 111.84, 115.14, 117.0, 130.23, 147.86, 155.35, 158.14, 160.40; EI-Mass: 304.8 ( $\text{M}^+ - 1$ );

**5-Nitro-furan-2-carboxylic acid 3,4-dimethoxy-benzylamide (23).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and 3,4-dimethoxybenzylamine (289  $\mu\text{L}$ , 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general

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procedure to afford 526 mg of product (90% yield). TLC:  $R_f$  0.3 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$ 3.9 (6Hs, s) 4.19 (2Hs, d,  $J$  = 6.5 Hz), 6.8-6.97 (3Hs, m), 7.31 (1H, d,  $J$  = 3.4 Hz), 7.39 (1H, d,  $J$  3.4 Hz);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 42.91, 55.39, 55.40, 110.85, 111.02, 111.87, 115.46, 119.99, 129.11, 147.53, 148.31, 148.73, 155.59; EI-Mass: 205.0 ( $\text{M}^+ - 1$ ).

**5-Nitro-furan-2-carboxylic acid 3,4,5-trimethoxy-benzylamide (24).**

5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and 3,4,5-trimethoxy-benzylamine (326  $\mu\text{L}$ , 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 532 mg of product (83% yield). TLC:  $R_f$  0.80 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$ 3.87 (3Hs, s), 3.89 (6Hs, s), 4.58 (2Hs, d,  $J$  = 5.8 Hz), 6.59 (2Hs, s), 6.86-6.93 (1H, bs), 7.32 (1H, d,  $J$  = 4.2 Hz), 7.4 (1H, d,  $J$  = 4.2 Hz);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 43.42, 55.65, 60.28, 104.85, 111.88, 115.59, 132.13, 147.42, 153.01, 155.59; EI-Mass: 335.0 ( $\text{M}^+ - 1$ ).

**5-Nitro-furan-2-carboxylic acid 1,2,3,4-tetrahydro-naphthalen-1-yl)-amide (25).**

5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and 1-amino(1,2,3,4-tetrahydro)naphthalene (274  $\mu\text{L}$ , 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol).

The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 388 mg of product (71% yield). TLC:  $R_f$  0.75 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$ 1.8-2.22 (4Hs, m), 2.78-2.98 (2Hs, m), 5.33-5.45 (1H, m), 6.81-6.9 (1H, bd,  $J$  = 8.3 Hz), 7.14-7.27 (3Hs, m), 7.28 (1H, d,  $J$  = 3 Hz), 7.3 (1H, d,  $J$  = 4.1 Hz), 7.38 (1H, d,  $J$  = 4.1 Hz);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 19.41, 28.53, 29.48, 47.37, 111.90, 115.55, 125.58, 125.93, 126.46, 127.24, 128.12, 128.20, 128.44, 128.90; EI-Mass: 384.9 ( $\text{M}^+ - 1$ ).

**5-Nitro-furan-2-carboxylic acid indan-1-ylamide (26).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and 1-amino-indane (246  $\mu\text{L}$ , 1.9 mmol) in

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DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure afford 415 mg of product (80% yield). TLC:  $R_f$  0.75 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$ 1.95-2.1 (1H, m), 2.62-2.76 (1H, m), 2.88-3.02 (1H, m), 3.03-3.17 (1H, m), 5.67 (1H, q,  $J$  6.75 Hz, 13.5 Hz), 6.88-6.97 (1H, bd,  $J$  = 6.75 Hz), 7.22-7.39 (m6Hs, m);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 29.77, 33.14, 54.36, 111.9, 115.53, 123.64, 124.48, 126.45, 127.92, 141.42, 142.98, 147.51, 155.50; EI-Mass: 370.9 ( $\text{M}^+$ -1).

**5-Nitro-furan-2-carboxylic acid phenethyl-amide (27).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and phenethylamine (239  $\mu\text{L}$ , 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure afford 402 mg of product (81% yield). TLC:  $R_f$  0.70 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$ 2.95 (2Hs, t,  $J$  = 7.5 Hz), 3.72 (2Hs, q,  $J$  = 13.8 Hz, 7.5 Hz), 6.81-6.92 (1H, bs), 7.21-7.38 (7Hs, m);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 35.04, 40.29, 111.8, 115.26, 126.3, 128.17, 128.28, 137.58, 147.50, 155.68; EI-Mass: 258.8 ( $\text{M}^+$ -1).

**5-Nitro-furan-2-carboxylic acid [2-(4-methoxy-phenyl)-ethyl]-amide (28).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and 4-methoxy-phenethylamine (279  $\mu\text{L}$ , 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 443 mg of product (80% yield). TLC:  $R_f$  0.6 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$ 2.9 (2Hs, t,  $J$  = 7.1 Hz), 3.68 (2Hs, q,  $J$  = 14.2 Hz, 7.1 Hz), 3.81 (3Hs, s), 6.67-6.76 (1H, bs), 6.88 (2Hs, d,  $J$  = 8.6 Hz), 7.16 (2Hs, d,  $J$  = 8.6 Hz), 7.25 (1H, d,  $J$  = 3.8 Hz), 7.36 (1H, d,  $J$  = 3.8 Hz);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 34.12, 40.49, 54.73, 111.83, 113.69, 115.21, 129.12, 129.58, 147.58, 155.70, 157.97; EI-Mass: 288.8 ( $\text{M}^+$ -1).

**5-Nitro-furan-2-carboxylic acid (1-phenyl-ethyl)-amide (29).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and 1-(S)-phenyl-ethylamine (245

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$\mu$ L, 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 422 mg of product (85% yield). TLC:  $R_f$  0.75 (1:1 hexane:ethyl acetate);  
5  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.65 (3Hs, d,  $J = 7.2$  Hz), 5.32 (1H, quin,  $J = 14.0$  Hz, 7.2 Hz), 6.8-6.92 (1H, bd,  $J = 7.2$  Hz), 7.24-7.45 (7Hs, m);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 20.92, 48.69, 111.92, 115.54, 125.77, 127.31, 128.33, 141.44, 147.52, 154.84; EI-Mass: 258.8 ( $\text{M}^+ - 1$ ).

**5-Nitro-furan-2-carboxylic acid (1-phenyl-ethyl)-amide (30).** 5-Nitro-  
10 2-furan carboxylic acid (300 mg, 1.9 mmol) and 1-(R)-phenyl-ethylamine (245  $\mu$ L, 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 422 mg of product (85% yield). TLC:  $R_f$  0.75 (1:1 hexane:ethyl acetate);  
15  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.65 (3Hs, d,  $J = 7.2$  Hz), 5.32 (1H, quin,  $J = 14.0$  Hz, 7.2 Hz), 6.8-6.92 (1H, bd,  $J = 7.2$  Hz), 7.24-7.45 (7Hs, m);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 20.91, 48.69, 111.93, 115.54, 125.77, 127.31, 128.32, 141.47, 147.53, 154.87; EI-Mass: 258.8 ( $\text{M}^+ - 1$ ).

**5-Nitro-furan-2-carboxylic acid [2-(3,4-dimethoxy-phenyl)-ethyl]-amide (31).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and 2,4-dimethoxy phenethylamine (319  $\mu$ L, 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 550 mg of product (90% yield). TLC:  $R_f$  0.75 (1:1  
20 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.89 (2Hs, t,  $J = 7.3$  Hz), 3.69 (2Hs, q,  $J = 14.7$  Hz, 7.3 Hz), 3.86 (3Hs, s), 3.88 (3Hs, s), 6.7-6.87 (4Hs, m), 7.24 (1H, d,  $J = 4$  Hz), 7.35 (1H, d,  $J = 4$  Hz);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 34.58, 40.39, 55.36, 55.41, 111.06, 111.37, 111.83, 115.23, 120.15, 130.10, 147.45, 147.55, 148.71, 155.68; EI-Mass: 318.9 ( $\text{M}^+ - 1$ ).

**5-Bromo-furan-2-carboxylic acid 4-methoxy-benzylamide (32).** 5-Bromo-2-furan carboxylic acid (**7a**) (360 mg, 1.9 mmol) and 4-methoxy

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bezylamine (249  $\mu$ L, 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 538 mg of product (90% yield). TLC:  $R_f$  0.75 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$ 3.82 (3Hs, s), 4.55 (2Hs, d,  $J$  = 6.2 Hz), 6.45 (1H, d,  $J$  = 3.6 Hz), 6.5-6.6 (1H, bs), 6.9 (2Hs, d,  $J$  = 5.3 Hz), 7.1 (1H, d,  $J$  = 3.6 Hz), 7.29 (2Hs, d,  $J$  = 5.3 Hz);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 42.19, 54.76, 113.57, 113.61, 116.08, 123.81, 128.79, 129.39, 149.01, 156.54, 158.63; EI-Mass: 333.9 ( $\text{M}^+$ +23).

**5-Bromo-furan-2-carboxylic acid (3-methoxy-phenyl)-amide (33).** 5-Bromo-2-furan carboxylic acid (500 mg, 2.6 mmol) and m-anisidine (292  $\mu$ L, 2.6 mmol) in DMF (10 mL) was treated with EDCI (993 mg, 5.2 mmol) followed by DMAP (793 mg, 6.5 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure afforded 606 mg of product (78% yield). TLC:  $R_f$  0.80 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$ 3.85 (3Hs, s), 6.51 (1H, d,  $J$  = 4.0 Hz), 6.72 (1H, dd,  $J$  = 8.1 Hz, 2.7 Hz), 7.12 (1H, d,  $J$  = 8.1 Hz), 7.18 (1H, d,  $J$  = 4.0 Hz), 7.26 (1H, t,  $J$  = 8.1 Hz), 7.43 (1H, t,  $J$  = 2.7 Hz), 8.0-8.1 (1H, bs);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 54.71, 105.48, 110.07, 111.98, 113.98, 116.92, 124.42, 129.15, 137.92, 148.79, 154.61, 159.56; EI-Mass: 293.6 ( $\text{M}^+$ -1).

**5-Sulfo-furan-2-carboxylic acid (9a).** A solution of  $\text{AgNO}_3$  (1.7 g, 10 mmol) in 5 mL of water was added with stirring to a solution of NaOH (0.8 g, 20 mmol) in 5 mL of water. Sodium,5-formyl-furan-2-sulfonate (**8a**) (1 g, 5 mmol) was added in portions to the resulting brown mixture. The reaction mixture was stirred for 0.5 hr. at room temperature, filtered and the residue was washed with 10 mL of hot water. The chilled filtrate was neutralized with con. HCl and the product was used as such in further reactions.  $^1\text{H}$  NMR (300 MHz,  $\text{D}_2\text{O}$ ):  $\delta$ 7.1 (1H, d,  $J$  = 4.5 Hz), 7.22 (1H, d,  $J$  = 4.5 Hz);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 112.91, 115.34, 149.42, 152.06, 164.69; EI-Mass: 190.6 ( $\text{M}^+$ -1).

**5-(3-Methoxy-phenylcarbamoyl)-furan-2-sulfonic acid (34).** 5-Sulfo-furan-2-carboxylic acid (**9a**) (191 mg, 1 mmol) and m-anisidine (122  $\mu$ L, 1

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mmol) in DMF (5 mL) was treated with EDCI (382 mg, 2 mmol), DMAP (30 mg, 0.25 mmol), NEt<sub>3</sub> (388  $\mu$ L, 3 mmol) and followed the reaction as explained above to afford 112 mg of product (38% yield). TLC: R<sub>f</sub> 0.40 (20:1 chloroform:methanol); <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O):  $\delta$ 3.61 (3Hs, s), 6.58 (1H, dd, J = 8.2 Hz, 2.7 Hz), 6.81 (1H, d, J = 3.5 Hz), 6.92 (1H, dd, J = 8.2 Hz, 1.6 Hz), 6.98 (1H, t, J = 2.7 Hz), 7.03 (1H, d, J 3.5 Hz), 7.11 (1H, t, J = 8.2 Hz), 7.68 (1H, d, J = 8.1 Hz); <sup>13</sup>C NMR (300 MHz, CDCl<sub>3</sub>): 54.64, 105.38, 106.29, 109.94, 111.20, 112.0, 114.69, 129.03, 138.19, 138.43, 146.70, 159.42; EI-Mass: 296.2 (M<sup>+</sup>-1).

**5-Nitro-furan-2-carboxylic acid (3-hydroxy-phenyl)-amide (35).** 5-Nitro-2-furan carboxylic acid (300 mg, 1.9 mmol) and 3-amino-phenol (208 mg, 1.9 mmol) in DMF (5 mL) was treated with EDCI (730 mg, 3.8 mmol) followed by DMAP (582 mg, 4.7 mmol). The reaction mix was stirred for 14 hr. at room temperature and worked up as explained in general procedure to afford 331 mg of product (70% yield). TLC: R<sub>f</sub> 0.50 (1:1 hexane:ethyl acetate); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD):  $\delta$ 5.09 (1H, ddd, J = 8.0 Hz, 2.5 Hz, 1.0 Hz), 5.59 (1H, ddd, J = 8.0 Hz, 2.0 Hz, 1.0 Hz), 5.64 (1H, t, J = 8.0 Hz), 5.78 (1H, t, J = 2.0 Hz), 5.9 (1H, d, J = 4.0 Hz), 6.45 (1H, d, J = 4.0 Hz), 6.45 (1H, s); EI-Mass: 247.2 (M<sup>+</sup>-1).

**5-Phenylsulfanyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide (36).** Mixture of thiophenol (0.35 mL, 0.034 mmol) in DMF (5 mL) added NaH (0.24 g, 10.2 mmol) at 0°C, stirred for 15 min. Then slowly added 5-Bromo-furan-2-carboxylic acid (3-methoxy-phenyl)-amide **33** (1 g, 0.34 mmol) and stirring continued for 12 hr. at 150°C. Reaction mixture was treated with sat. NH<sub>4</sub>Cl (3 mL), diluted with water (50 mL) and extracted with EtOAc (3 x 50 mL). The combined EtOAc fractions was washed with brine (75 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in a vacuum followed by flash column purification with Pet. Ether and EtOAc in 2:1 ratio, to give 884 mg of product (80% yield). TLC: R<sub>f</sub> 0.80 (1:1 hexane:ethyl acetate); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$ 3.88 (3Hs, s), 6.76 (1H, dd, J = 8 Hz, 2 Hz), 6.88 (1H, d, J = 3.5 Hz), 7.16 (1H, dd, J = 8 Hz, 1.5 Hz), 7.28-7.35 (4Hs, m), 7.36-7.41 (2Hs, m), 7.47 (1H, t, J = 2.5

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Hz), 8.04-8.11 (1H, bs);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 54.76, 105.35, 110.23, 111.89, 116.39, 120.37, 126.78, 128.1, 128.91, 129.19, 135.01, 137.84, 146.38, 149.89, 154.99, 159.62; EI-Mass: 348.3 ( $\text{M}^+ + 23$ ).

**5-Nitro-furan-2-carboxylic acid (3-benzyloxy-phenyl)-amide (37).**

5 Compound **35** (150 mg, 0.6 mmol) was dissolved in dry THF (5 mL) and  $\text{K}_2\text{CO}_3$  (167 mg, 1.2 mmol) followed by benzyl bromide (146  $\mu\text{L}$ , 1.2 mmol). The reaction mixture was stirred for 12 hr. at room temperature. The reaction mixture was diluted with 30 mL ethyl acetate and washed with  $\text{H}_2\text{O}$  (25 mL),  
brine (25 mL). The ethyl acetate was dried and concentrated. The crude  
10 product was purified with flash column using 15% EtOAc in hexane to afford 147 mg of product (72% yield). TLC:  $R_f$  0.82 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$ 5.16 (2Hs, s), 6.9 (1H, dd,  $J = 8.3, 2.4, 1.0$  Hz), 7.23 (1H, dd,  $J = 8.1, 2.0, 0.8$  Hz), 7.35 (1H, t,  $J = 8$  Hz), 7.39 (1H, dt,  $J = 7.0, 2.5$  Hz), 7.42–7.44 (4Hs, m), 7.5–7.52 (2Hs, m), 7.56 (1H, t,  $J = 2.2$  Hz), 8.26–8.3  
15 (1H, bs);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 69.59, 106.51, 111.82, 112.1, 112.26, 116.24, 126.94, 127.5, 128.06, 129.48, 136.15, 136.99, 147.28, 153.37, 158.93; EI-Mass: 337.6 ( $\text{M}^+ - 1$ ).

**5-Benzenesulfinyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide (38) and 5-Benzenesulfonyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide (39).** A mixture of compound **36** (0.1 g, 0.3 mmol) and  $\text{NaHCO}_3$  (0.116 g, 1.3 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL) at  $0^\circ\text{C}$  treated with *m*-chloroperbenzoic acid (0.116 g, 0.67 mmol) and stirred for 3 hr. The reaction mixture was quenched with dil. Aq.  $\text{NH}_4\text{OH}$  solu. (5 mL) and diluted with  $\text{CH}_2\text{Cl}_2$  (30 mL). The organic layer was quenched with dil. Aq.  $\text{NH}_4\text{OH}$  solu. (30 mL),  
20 water (30 mL), brine (30 mL) and dried over  $\text{Na}_2\text{SO}_4$ . The organic solution was concentrated in vacuum followed by flash column purification with Pet. Ether and EtOAc in 5:1 ratio afforded the products 31 mg of **38** and 38 mg of **39** in 30% and 35% yields respectively. **5-Benzenesulfinyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide (38):** TLC:  $R_f$  0.30 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$ 4.87 (3H, s), 6.78 (1H, dd,  $J = 8$  Hz, 2.5 Hz), 6.81 (1H, d,  $J = 3.5$  Hz), 7.15 (1H, dd,  $J = 8$  Hz, 1.5 Hz), 7.29 (1H, d,  $J = 3.5$  Hz),  
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7.32 (1H, d, J = 8 Hz), 7.42 (1H, t, J = 2 Hz), 7.64 (3Hs, t, J = 3 Hz), 7.82 (2Hs, dd, J = 6Hz, 3.5 Hz, 2.5 Hz), 8.2 (1H, s);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 54.81, 105.30, 110.49, 111.77, 115.16, 116.54, 124.47, 129.07, 129.29, 131.50, 137.45, 140.35, 150.73, 154.32, 159.68, EI-Mass: 340.6 ( $\text{M}^+ - 1$ ); **5-Benzenesulfonyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide (39):** TLC:  $R_f$  0.70 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  4.85 (3H, s), 6.79 (1H, dd, J = 8Hz, 2.2 Hz, 1.7 Hz), 7.18 (1H, dd, J = 7.4 Hz, 1.5 Hz), 7.28-7.34 (3Hs, m), 7.4 (1H, t, J = 2.5 Hz), 7.62 (2Hs, t, J = 8 Hz), 7.72 (1H, t, J = 7 Hz), 8.09 (2Hs, d, J = 7 Hz), 8.22 (1H, s);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 54.81, 105.57, 110.66, 112.02, 115.29, 118.42, 127.47, 129.09, 129.3, 133.85, 137.22, 138.65, 150.34, 150.63, 154.07, 159.67; EI-Mass: 356.5 ( $\text{M}^+ - 1$ ).

**5-(3-Methoxy-phenylcarbamoyl)-furan-2-carboxylic acid ethyl ester (40).** A flame evacuated three neck round bottom flask fitted with reflux condenser, containing magnesium (32 mg, 1.3 mmol) under argon atmosphere was added dry THF (3 mL), followed by 5-Bromo-furan-2-carboxylic acid (3-methoxy-phenyl)-amide **33** (0.2 g, 0.67 mmol) in 2 mL THF and ethyl bromide (72 mg, 0.67 mmol). The resulting mixture was stirred for 15 min. at 50°C and cooled to 0°C, then added ethyl chloroformate (0.145 g, 1.3 mmol), continued stirring at room temperature for 5 hr. Then aq. Sat. $\text{NH}_4\text{Cl}$  solu. (1 mL) was added to the reaction mixture and diluted with EtOAc (50 mL). The EtOAc was washed with brine (50 mL) dried over  $\text{Na}_2\text{SO}_4$ , concentrated under vacuum and purified by flash-column to give product only 39 mg (20% yield). TLC:  $R_f$  0.75 (1:1 hexane:ethyl acetate);  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.21 (3Hs, t, J = 8.6 Hz), 3.56 (3Hs, s), 4.25 (2Hs, q, J = 8.6 Hz, 14.14 Hz), 6.51 (1H, dd, J = 1.69 Hz, 3.38 Hz), 6.81 (1H, t, J = 1.69 Hz), 6.87 (1H, ddd, J = 0.65 Hz, 2.6 Hz, 7.54 Hz), 6.92 (1H, ddd, J = 0.65 Hz, 2.6 Hz, 7.54 Hz), 7.03 (1H, dd, J = 0.65 Hz, 3.38 Hz), 7.33 (1H, t, J = 7.54 Hz), 7.55 (1H, dd, J = 0.65 Hz, 1.69 Hz);  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ): 13.47, 54.87, 62.80, 111.69, 113.15, 113.5, 118.29, 119.66, 129.27, 138.74, 145.12, 159.68, 160.01; EI-Mass: 312.1 ( $\text{M}^+ + 23$ ).

**Preparation of 5-Nitro-furan-2-carbonyl chloride (6a).** 5-Nitro-furan-2-carboxylic acid (942 mg, 6 mmol) in DCM (10 mL) was treated with

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oxalylchloride (1.046 mL, 12 mmol) followed by 2 drops of DMF and stirred at room temperature for 4 hr. The reaction mix was concentrated in vacuum to obtain acid chloride.

**General procedure for preparation of amides (6a).**

5        Method 1: 5-Nitro-furan-2-carbonyl chloride (526 mg, 3 mmol) in DMF (5 mL) was added to amine (3 mmol) in pyridine (5 mL) and reaction was carried out at 60°C. The reaction was diluted with EtOAc (100 ml), washed with 10% aqueous NaHCO<sub>3</sub> (2 x 50 ml), water (2 x 50 ml) and brine (2 x 50 ml). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated, followed by  
10       flash column purification, which provided corresponding amides.

Method 2: 5-Nitro-furan-2-carbonyl chloride (930 mg, 5.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 ml) was added amine (5.3 mmol, 1 equiv.) in Et<sub>3</sub>N (3 ml) and the mixture was stirred for 14 hrs. at 47°C. Reaction was followed by TLC, after completion of reaction 100 ml of EtOAc was added and washed with 10%  
15       aqueous NaHCO<sub>3</sub> (2 x 50 ml), water (2 x 50 ml) and brine (2 x 50 ml). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated followed by flash column purification, which provided corresponding amides.

**5-Nitro-furan-2-carboxylic acid pyrazin-2-ylamide (48).** 5-Nitro-furan-2-carbonyl chloride (526 mg, 3 mmol) in DMF (5 ml) was added aminopyrazine  
20       (285 mg, 3 mmol) followed by pyridine (5 ml). The reaction was stirred for 14 hr. at 60°C. The reaction was followed as explained in method 1 above to yield 120 mg (17%) of compound **48**; TLC: R<sub>f</sub> 0.30 (1:1 hexane:ethylacetate). <sup>1</sup>HNMR (500 MHz, CDCl<sub>3</sub>): δ 7.46 (1H, d, J = 3.91 Hz), 7.49 (1H, d, J = 3.91 Hz), 8.37-8.40 (1H, m), 8.49 (1H, d, J = 2.44 Hz), 8.76-8.86 (1H, bs), 9.65 (1H, d, J = 1.47 Hz); <sup>13</sup>CNMR (300 MHz, mixture of CD<sub>3</sub>OD:CDCl<sub>3</sub> 1:3): 111.90, 117.33, 136.68, 140.00, 142.26; EI-Mass: 234.9 (M<sup>+</sup>1 232.9 (M<sup>+</sup>-1); IR: 1701, 3110, 3180 cm<sup>-1</sup>.  
25

**5-nitro-furan-2-carboxylic acid (pyridin-2-yl methyl)-amide (49).** To the mixture of 5-Nitro-furan-2-carbonyl chloride (930 mg, 5.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub>  
30       (10 ml) was added 2-aminomethylpyridine (0.54 ml, 5.3 mmol) in Et<sub>3</sub>N (3 ml) and stirred for 14 hrs. at 47°C. The reaction was followed as explained in

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method 2 to yield 1.12 gm (85 %) of product **49**. TLC:  $R_f$  0.11 (1:1 hexane:ethyl acetate);  $^1\text{H}$ NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$ 4.83 (2H, d,  $J$  = 5.37 Hz), 7.32 (1H, d,  $J$  = 3.91 Hz), 7.38 (1H, d,  $J$  = 3.66 Hz), 7.39-7.42 (1H, m), 7.48 (1H, d,  $J$  = 7.81 Hz); 7.86 (1H, dt,  $J$  = 1.71 Hz), 8.10-8.22 (1H, bs), 8.65 (1H, d,  $J$  = 4.8 Hz);  $^{13}\text{C}$ NMR (300 MHz,  $\text{CDCl}_3$ ): 43.75, 111.75, 121.55, 122.16, 136.40, 147.60, 148.70, 154.70, 155.79; EI-Mass: 248 ( $M^+$ +23); IR: 1670, 3305  $\text{cm}^{-1}$ .

**5-nitro-furan-2-carboxylic acid (4-methoxy-benzothiazol-2-yl)-amide (50).** To the mixture of 5-Nitro-furan-2-carbonyl chloride (667 mg, 3.8 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 ml) was added 2-amino 4-methoxy benzothiazole (684 mg, 3.8 mmol) followed by pyridine (5 ml) and the reaction mixture was stirred for 14 hr. at room temperature. The reaction was followed as explained in method 2 to yield 480 mg (29%) of compound **50**. TLC:  $R_f$  0.37 (1:1 hexane:ethyl acetate).  $^1\text{H}$ NMR (500 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$ 4.03 (3H, s), 7.04 (1H, d,  $J$  = 8.06 Hz), 7.30 (1H, t,  $J$  = 8.06 Hz), 7.44 (1H, d,  $J$  = 8.06 Hz), 7.53 (1H, m), 7.59 (1H, d,  $J$  = 3.66 Hz);  $^{13}\text{C}$ NMR (300 MHz,  $\text{CDCl}_3$ ): 29.15, 55.36, 106.56, 111.59, 113.07, 117.70, 125.12; EI-Mass: 318 ( $M^+$ -1); IR: 1561, 1701  $\text{cm}^{-1}$ .

**5-nitro-furan-2-carboxylic acid (6-methoxy-pyrimin-4-yl)-amide (51).** To the mixture of 5-nitro-furan-2-carbonyl chloride (667 mg, 3.8 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 ml) was added 4-amino 6-methoxy pyrimidine (475 mg, 3.8 mmol) followed by pyridine (5 ml) and reaction was stirred for 14 hr. at 50°C. The reaction was followed as explained in method 2 to yield 550 mg (40%) of compound **51**. TLC:  $R_f$  0.53 (1:1 hexane:ethyl acetate).  $^1\text{H}$ NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$ 4.04 (3H, s), 7.44 (2H, q,  $J$  = 4.04, 8.06 Hz), 7.65 (1H, d,  $J$  = 0.97 Hz), 8.58 (1H, d,  $J$  = 0.98 Hz), 8.85 (1H, s);  $^{13}\text{C}$ NMR (300 MHz,  $\text{CDCl}_3$ ): 53.77, 95.60, 111.79, 117.34, 145.98, 154.13, 156.21, 170.80; EI-Mass: 263 ( $M^+$ -1); IR: 1576, 1684, 3123  $\text{cm}^{-1}$ .

**5-nitro-furan-2-carboxylic acid 2-methoxy-benzylamide (52).** To the mixture of 5-nitro-furan-2-carbonyl chloride (877 mg, 5 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 ml) was added to 2-methoxy benzyl amine (0.646 ml, 5 mmol) in  $\text{Et}_3\text{N}$  (3 ml) and reaction was stirred for 14 hr. at room temperature. The reaction was followed as explained in method 2 to yield 685 mg (49%) of compound **52**.  $R_f$  0.53 (1:1

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hexane:ethyl. acetate).  $^1\text{H}$ NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$ 3.94 (3H, s), 4.65 (2H, d,  $J$  = 5.86 Hz), 6.96 (2H, dd,  $J$  = 7.09, 7.32 Hz), 10-7.20 (1H, bs), 7.26 (1H, d,  $J$  = 3.66 Hz), 7.31-7.36 (3H, m);  $^{13}\text{C}$ NMR (300 MHz,  $\text{CDCl}_3$ ): 38.94, 54.88, 109.91, 109.98, 111.87, 115.19, 120.18, 124.52, 128.79, 129.33, 147.81, 155.46, 157.07; EI-Mass: 299.3 ( $\text{M}^+ + 23$ ); IR: 1676, 3307  $\text{cm}^{-1}$ .

**5-nitro-furan-2-carboxylic acid 2,3-dimethoxy-benzylamide (53).** To the mixture of 5-nitro-furan-2-carbonyl chloride (877 mg, 5 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 ml) was added to 2,3-dimethoxy benzyl amine (0.734 ml, 5 mmol) in  $\text{Et}_3\text{N}$  (3 ml) and reaction was stirred for 14 hr. at room temperature. The reaction was followed as explained in method 2 to yield 830 mg (54%) of compound **53**.  $R_f$  0.48 (1:1 hexane:ethyl acetate).  $^1\text{H}$ NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$ 3.90 (3H, s), 3.95 (3H, s) 4.65 (2H, d,  $J$  = 6.10 Hz), 6.92 (2H, dd,  $J$  = 1.46, 7.05 Hz), 6.95 (1H, dd,  $J$  = 1.46, 7.81 Hz), 7.03-7.09 (3H, m);  $^{13}\text{C}$ NMR (300 MHz,  $\text{CDCl}_3$ ): 38.91, 55.21, 60.19, 111.88, 115.27, 120.84, 123.63, 130.04, 146.76, 147.66, 152.11, 155.54; EI-Mass: 329 ( $\text{M}^+ + 23$ ), 305 ( $\text{M}^+ - 1$ ); IR: 1671, 3323  $\text{cm}^{-1}$ .

### Biological Results

To develop the structure activity relationship of **1**, 43 compounds were synthesized and tested for enzymatic inhibition of Glf and for MIC activity against *M. tuberculosis* (Table 1). Although most of the tested compounds inhibited Glf, the best inhibitors of Glf were **10** and **13**, both 3-substituted anilinylnitrofuranyl amides. In general substituted nitrofuranyl benzylamides, were less active than the analogous anilinylnitrofuranyl amides. The nitrofuranyl group was shown to be required for Glf activity.

The MIC of the nitrofuranyl amides against *M. tuberculosis* H37Ra was determined by the micro broth dilution method using microtiter plates. *M. tuberculosis* was grown in Middlebrook 7H9 medium to an  $\text{OD}^{650}$  of 0.4 - 0.6 and a dilution made to an  $\text{OD}_{650}$  of 0.01. 100  $\mu\text{l}$  of these cells are then added to microtiter well containing serial dilutions of the nitrofuranyl amides. The cell are then incubated at 37°C for 7 days and visually examined for growth.  $\text{MIC}_{90}$  was determined for wells with greater than 90% inhibition of growth.

Cytotoxicity was determined using alamar blue assay against Vero cells.

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An assay for Maximum Tolerated Dose (MTD) was also utilized. Three healthy mice were given orally one single dose of the compound and are observed at regular times for any adverse effects. Three different concentrations are tested, generally at 100, 300 and 500 mg/kg. The latter dose is about twice to five times the dose used for efficacy testing of the compound in mice. After 7 days of observation the mice are sacrificed and the organs are studied for any adverse effects. In case of abnormalities the organs are fixed in formalin and given for extensive pathology analysis.

Mice used as a GKO mouse model were infected via low dose aerosol to reproducibly deliver *M. tuberculosis* in the alveolar regions of the lungs in low numbers to mimic the realistic disease in humans. Treatment was initiated 18 days postinfection for 9 daily treatments for one single dose (at 300 mg/kg). Bacterial load was determined 28 days postinfection in lungs and spleens of the mice. To determine whether the results have statistical significant value, statistics on the data for every compound were performed using the SigmaStat™ program. Due to the short term treatment regimen, fluctuations in CFU within mice from one treatment group are limited and a reduction of ~0.3 Log10 CFU in the lungs is considered statistically significant.

The MIC activity of the series showed a strong structure activity relationship with the nitro group being required for activity in all cases. Aniliny, benzyl and phenethyl amides all had significant activity, with increased activity compared to saturated cyclohexylamide **15** and adamantylamide amide **16**. Heteroaromatic substitutions such as pyridines **44**, **45**, **46**, pyrazole **47**, pyrazine **48**, furfuryl amide **18** all were less active than the corresponding aniline amide **17**. Tertiary amides **41**, **42** were less active than their corresponding secondary amides **14**, **17**. The most active series was the methoxy substituted benzylamides with a range of relative activities 4-methoxybenzyl **20** > 3,4,-dimethoxy benzyl **23** > 2,4-dimethoxy benzyl **22** > 3,4,5-trimethoxy benzyl **24** > 2,3 dimethoxy benzyl **53** > 2-methoxy benzyl **52**. The activity of this series shows a clear preference for 4-methoxy substituted systems. Compounds in the methoxy benzyl series showed the highest

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therapeutic index principally due to their low MIC values. The nitrofuranyl amides when tested against other bacteria *Mycobacterium smegmatis*, *Staphylococcus aureus* or *Escherichia coli* in MIC assays were all inactive.

5 Added to Table 1 are the predictive pharmacokinetic values of CLogP, Solubility and Protein binding. These values were used to aid the decision as to which compounds were to advance towards *in vivo* testing. Volsurf was used to predict the solubility and protein binding. The predicted values for protein binding are in percent protein bound. An ideal antimicrobial agent would have a low percent protein bound prediction, as protein binding can  
10 influence such factors as delivery to target tissue, effective MIC concentration in human serum, drug interactions, metabolism, and clearance. The predicted protein binding values for this series is acceptable.

After examining MIC, therapeutic index, Glf inhibition, CLogP, calculated solubility and protein binding data for the compound series, compounds **10**, **12**,  
15 **13**, **20**, **23** were selected for *in vivo* testing.

Maximum tolerated dosing was performed on these selected compounds. An escalating dose of drug (100, 300 and 500 mg/kg) was given to mice by oral gavage. Compounds **10**, **12** and **23** showed no effect at the maximum dose. **13** showed some pathology at 500 mg/kg.

20 Subsequently, the four compounds were tested for efficacy against *M. tuberculosis* at a dose (see Table 1) lower than the MTD in infected C57BL/6 Interferon- $\gamma$  gene depleted mice (see below). The results of the experiment are presented in Table 2 below.

The sub-microgram MIC activity of some of the nitrofuranyl amides has  
25 lead to the exploration of their usage as anti-tuberculosis agents. The MIC activity of this series compares well with front-line anti-tuberculosis agents such as isoniazid (MIC<sub>90</sub> 0.05  $\mu$ g/mL) and ethambutol (MIC<sub>90</sub> 0.78  $\mu$ g/mL) and they have an acceptable therapeutic index (Table 1). Four compounds passed the maximum tolerated dose assay and compound **23** showed significant oral  
30 activity against *M. tuberculosis* in the mouse infection model.

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Like PA824, their activity is restricted to mycobacteria of the mycobacterium tuberculosis complex with no appreciable activity against *M. smegmatis*, *S. aureus* or *E. coli*, a property that believed to be desirable for the development of new tuberculosis treatments.

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Compound	R <sub>1</sub>	HNR <sub>2</sub> R <sub>3</sub>	TB MIC ( $\mu$ g/mL)	ToxIC <sub>50</sub>	Therap. Index <sup>2</sup>	CLogP <sup>b</sup>	Solub. <sup>c</sup>	Protein Binding <sup>d</sup>
1	NO <sub>2</sub>	3-chloro-4-methoxy aniline	1.6			2.554	-3.50	61.91
10	NO <sub>2</sub>	3-chloro-aniline	0.38	6.86	8.6	2.804	-3.52	68.45
11	NO <sub>2</sub>	3-bromo-aniline	1.6	15.37	9.6	2.954	-3.59	68.91
12	NO <sub>2</sub>	3-fluoro-aniline	0.8	9.29	11.6	2.234	-3.30	66.61
13	NO <sub>2</sub>	3-anisidine	0.8	18.63	23.3	1.975	-3.39	63.60
14	NO <sub>2</sub>	4-anisidine	0.4	21.44	53.6	1.975	-3.44	63.09
15	NO <sub>2</sub>	cyclohexylamine	3.1	9.08	2.9	2.243	-3.00	64.78
16	NO <sub>2</sub>	adamantyl amine	3.1	9.43	3.0	2.871	-3.57	74.18
17	NO <sub>2</sub>	aniline	0.8	2.19	2.7	2.001	-3.18	67.46
18	NO <sub>2</sub>	furfuryl amine	6.25	4.08	0.7	1.356	-2.92	62.01
19	NO <sub>2</sub>	4-amino-benzonitrile	0.8	3.53	4.4	1.342	-4.46	75.07
20	NO <sub>2</sub>	4-methoxy-benzylamine	0.1	16.36	163.6	2.099	-3.72	65.28
21	NO <sub>2</sub>	2-chloro-benzylamine	1.6	11.75	7.3	2.893	-3.36	68.69
22	NO <sub>2</sub>	2,4-dimethoxy-benzylamine	0.4	5.38	13.5	2.188	-3.65	59.13
23	NO <sub>2</sub>	3,4-dimethoxy-benzylamine	0.2	18.17	90.9	1.838	-3.77	63.68
24	NO <sub>2</sub>	3,4,5-trimethoxy-benzylamine	0.8	23.59	29.5	1.480	-4.12	65.57
25	NO <sub>2</sub>	1-amino-1,2,3,4-tetrahydro-naphthalene	3.1	9.03	2.9	3.153	-3.77	77.18
26	NO <sub>2</sub>	1-amino-indane	3.1	10.28	3.3	2.594	-3.65	77.02
27	NO <sub>2</sub>	phenethylamine	1.6	25.23	15.7	2.309	-3.76	75.35
28	NO <sub>2</sub>	4-methoxy-phenethylamine	0.8	20.45	25.6	2.228	-4.19	71.20
29	NO <sub>2</sub>	(S)-1-phenyl-ethylamine	1.6	25.63	16.0	2.489	-3.64	74.10



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30	NO <sub>2</sub>	(R)-1-phenyl-ethylamine	3.1	23.59	7.6	2.489	-3.44	71.13
31	NO <sub>2</sub>	3,4-dimethoxy-phenethylamine	0.4	27.44	68.6	1.967	-4.31	69.68
32	Br	4-methoxy-benzylamine	200	90.54	0.5	2.989	-3.85	69.41
33	Br	3-anisidine	100	61.19	0.6	2.864	-3.24	65.25
34	SO <sub>3</sub> H	3-anisidine	200			-0.886	-2.51	18.14
35	NO <sub>2</sub>	3-amino-phenol	1.6	12.5	7.8	1.334	-3.20	60.94
36	SPH	3-anisidine	25	25.69	1.0	4.303	-4.58	84.77
37	NO <sub>2</sub>	3-benzyloxy-aniline	12.5			3.743	-5.15	87.00
38	SOPh	3-anisidine	6.25	65	10.4	2.376	-4.25	61.04
39	SO <sub>2</sub> Ph	3-anisidine	6.25	25.85	4.1	2.736	-4.11	51.50
40	CO <sub>2</sub> Et	3-anisidine	400	200	0.5	2.665	-3.87	64.29
41	NO <sub>2</sub>	N-methyl-aniline	3.12			1.457	-2.94	66.83
42	NO <sub>2</sub>	N-methyl-4-anisidine	6.25			1.406	-3.15	60.45
43	NO <sub>2</sub>	2,3-dihydro-indole	0.8			3.249	-3.52	75.02
44	NO <sub>2</sub>	2-amino pyridine	3.12			1.051	-3.12	56.21
45	NO <sub>2</sub>	3-amino pyridine	6.25			1.051	-2.83	55.33
46	NO <sub>2</sub>	4-amino pyridine	3.12			1.051	-2.92	55.36
47	NO <sub>2</sub>	3-amino pyrazole	6.25			0.601	-2.97	48.70
48	NO <sub>2</sub>	2-amino pyrazine	6.25			0.286	-2.90	47.18
49	NO <sub>2</sub>	2-amino methyl pyridine	0.8			0.683	-3.02	57.31
50	NO <sub>2</sub>	2-amino-4-methoxy-benzothiazole	1.6			2.801	-3.96	74.30
51	NO <sub>2</sub>	4-amino-6-methoxy-pyrimidine	1.6			1.226	-3.33	53.13
52	NO <sub>2</sub>	2-methoxy-benzylamine	1.6			2.099	-3.30	66.45
53	NO <sub>2</sub>	2,3-dimethoxy-benzylamine	1.2			1.838	3.46	64.36

Table 2  
Determination of viable *M. tuberculosis* in spleens and lungs of infected mice after a 8-day drug treatment regimen

Compound	Lungs $\pm$ SEM	Spleen $\pm$ SEM
Untreated controls	8.0 $\pm$ 0.17	6.7 $\pm$ 0.16
Isoniazid (25 mg/kg)	5.5 $\pm$ 0.21	3.2 $\pm$ 0.21
Metronidazole (150 mg/kg)	8.5 $\pm$ 0.18	6.9 $\pm$ 0.04
<b>10</b>		
(300 mg/kg)	8.3 $\pm$ 0.21	7.3 $\pm$ 0.12
<b>12</b>		
(300 mg/kg)	8.4 $\pm$ 0.24	7.0 $\pm$ 0.11
<b>13</b>		
(150 mg/kg)	8.0 $\pm$ 0.3	7.2 $\pm$ 0.07
<b>23</b>		
(300 mg/kg)	6.5 $\pm$ 0.25	6.0 $\pm$ 0.19

(SEM = standard error)

Example 2

5 Example 1 describes a novel set of nitrofuranyl amides with potent antituberculosis activity. Compounds in this series were easy to synthesize, had a good therapeutic index, were active against anaerobically grown bacilli and were not cross resistant with other clinically used anti mycobacterial drugs.

10 The compound 5-Nitro-furan-2-carboxylic acid 3,4-dimethoxy-benzylamide (**23**) shown in Table 3 below specifically had significant oral activity in a mouse model of tuberculosis infection, as demonstrated in Example 1. Very few compounds have been described in the art with this level of *in vitro* activity against tuberculosis.

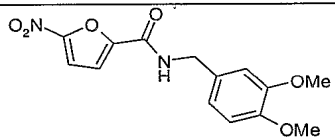
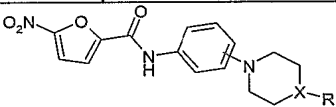
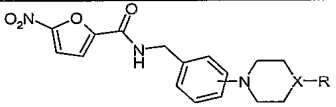
Without wishing to be limited by theory, it is hypothesized, based on analysis of these structures and their physical properties that oral bioavailability

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could potentially be limited in some cases for these compounds due to poor solubility and high crystal energy of the nitrofuranyl amide series. Such issues have been encountered in the development of other synthetic antimicrobial agents, most notably in the development of the fluoroquinolone class of antibiotics.

Therefore, Example 2 describes the synthesis and evaluation of related derivative compounds. These novel compounds are cyclic secondary amine substituted phenyl and benzyl nitrofuranyl amides (see Table 3) and are shown below to be effective novel anti tuberculosis agents.

The synthesis of these compounds is divided in to two classes: phenyl amides and benzyl amides to which were added a variety of cyclic secondary amines.

Table 3 Structures of Lead Compound and Amide Derivatives		
 <p>5-Nitro-furan-2-carboxylic acid 3,4-dimethoxy-benzylamide (<b>23</b>)</p>	 <p>phenyl amides</p>	 <p>benzyl amides</p>

### Methods and Materials

All the anhydrous solvents and starting materials were purchased from Aldrich Chemical Company (Milwaukee, Wisconsin, U.S.A.). All reagent grade solvents used for chromatography were purchased from Fisher Scientific (Suwanee, Georgia, U.S.A.) and FLASH<sup>TM</sup> column chromatography silica cartridges were obtained from Biotage Inc. (Lake Forest, Virginia, U.S.A.). The reactions were monitored by thin layer chromatography (TLC) on pre-coated Merck 60 F<sub>254</sub> silica gel plates and visualized using UV light (254 nm). Biotage FLASH 25+<sup>TM</sup> column chromatography system was used to purify mixtures. All <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker ARX-300 (300 and 75 MHz for <sup>1</sup>H and <sup>13</sup>C NMR, respectively; Billerica, Massachusetts, U.S.A.) or Varian INOVA-500<sup>TM</sup> (500 and 125 MHz for <sup>1</sup>H and <sup>13</sup>C NMR, respectively; Palo Alto,

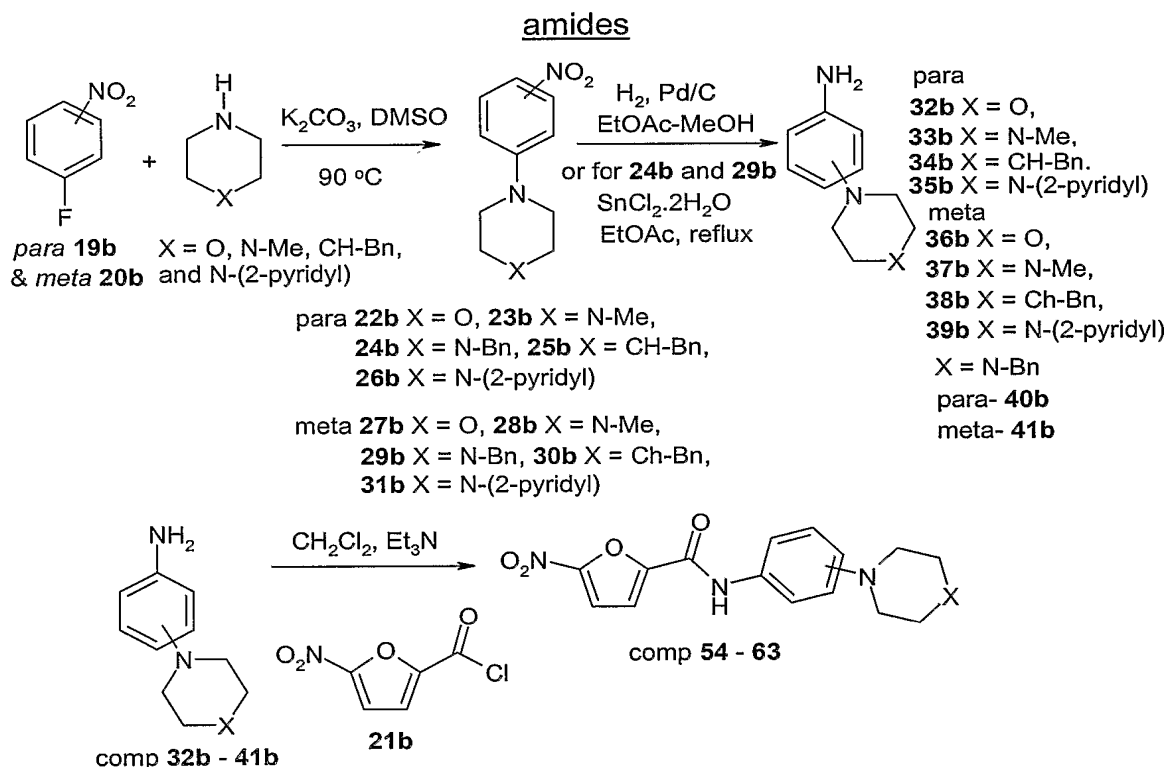
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California, U.S.A.) spectrometer. Chemical shifts are reported in ppm ( $\delta$ ) relative to residual solvent peak or internal standard (tetramethylsilane) and coupling constants ( $J$ ) are reported in hertz (Hz). Mass spectra were recorded on a Bruker ESQUIRE LCMS<sup>TM</sup> using ESI. Purity of the final products was confirmed before testing by analytical HPLC using a Alltech (Deerfield, Illinois, U.S.A.) platinum C-18 reverse phase column (4.5X150mm) and a H<sub>2</sub>O (0.1% TFA) to acetonitrile 0-100% linear gradient at a flow rate of 1.0 mL min<sup>-1</sup> and UV detection at 254nm.

#### Scheme 1

Referring now to Scheme 1, synthesis of the substituted phenyl amides involved a three reaction sequence of nucleophilic aromatic substitution, nitroreduction and acylation with the nitrofuranoic acid chloride. Accordingly, the fluorine of 3 or 4-fluoro nitrobenzene was substituted with secondary amides morpholine, 1-methyl-piperazine, 1-benzyl piperazine, 4-benzyl piperadine and 1-(2-pyridyl)piperazine to give corresponding substituted nitrobenzenes **22b** – **31b** with yields 78%-95%. The substitution on p-fluoro nitrobenzene was faster than meta-substitution 8 hours compared with 24 hours, respectively. The nitro functional group of compounds **22b** - **31b**, except compounds **24b** and **29b** were reduced by catalytic hydrogenation to give anilines **32b** - **39b** in quantitative yields. The amines **24b** and **29b** were reduced using SnCl<sub>2</sub>.2H<sub>2</sub>O to their corresponding amides **40b** - **41b** (both in 82% yields) due to sensitivity of the benzyl substituted piperazines to hydrogenation. Finally, all the amines **32b** - **39b** were treated with 5-nitro-furan-2-carbonyl chloride to give desired phenyl amides **54** - **63** in 82-90% yields.

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Scheme 1Synthesis of Cyclic Secondary Amine 3 or 4 Substituted Phenyl Nitrofuvaranyl

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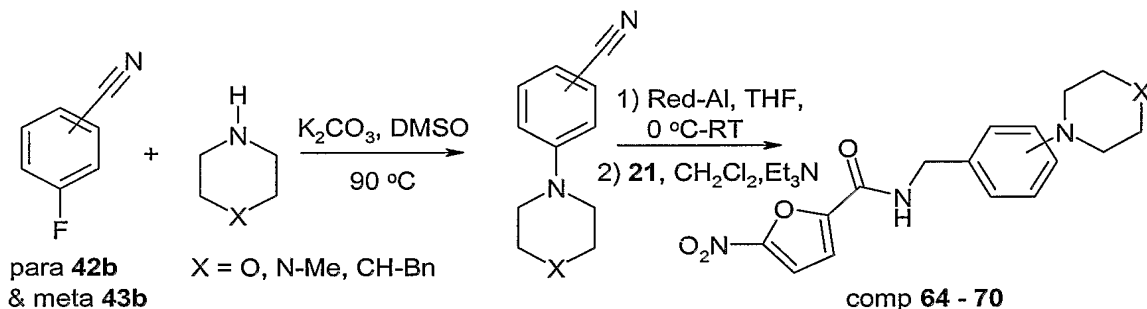
Scheme 2

Referring now to Scheme 2, the benzyl amide series was prepared by employing the similar pattern of reactions of nucleophilic aromatic substitution, reduction followed by acylation. In this case a cyano group was used as electron withdrawing group to facilitate the substitution. Accordingly, the fluorine of the 3 or 4-fluoro benzonitrile was substituted with corresponding cyclic secondary amines in DMSO at 90 °C and in the presence of potassium carbonate to give compounds **42b** - **48b** in a 83-96% yield. The substituted benzonitriles are subjected to reduction using Red-Al reagent to afford corresponding crude amines, which are further treated without purification with 5-nitro-furan-2-carbonyl chloride to give benzyl amides **64** - **70** in 69-86% yields.

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**Scheme 2****Cyclic secondary amine 3 or 4 substituted benzyl nitrofuranyl amides**

para **44b** X = O, **45b** X = N-Me  
**46b** X = N-Bn, **47b** X = CH-Bn  
 meta **48b** X = N-Me,  
**49b** X = N-Bn, **50b** X = CH-Bn

**General procedure for preparation of 22b - 31b and 44b – 50b.** To a mixture of substituted fluoro benzene (1 eq.) and K<sub>2</sub>CO<sub>3</sub> (1.5 eq.) in dimethyl sulfoxide (7 mL/g) was added secondary amine (2 eq.). The reaction mixture was stirred at 90° C and followed by TLC. After completion of reaction, the mixture was diluted with ethyl acetate (60 mL/g), and washed with water (2 x 50 mL/g), followed by brine (50 mL/g). The ethyl acetate fraction was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The crude products were purified by flash column chromatography to afford pure products.

**4-(4-Nitro-phenyl)-morpholine (22b).** To a mixture of 4-fluoro nitro benzene **19b** (425mg, 3.01 mmol) and K<sub>2</sub>CO<sub>3</sub> (623 mg, 4.52 mmol) in dimethyl sulfoxide (3 mL) was added morpholine (0.52 mL, 6.02 mmol) and the reaction continued as described above to afford amine 595 mg of **22b** in 95% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 3.37 (4Hs, t, J = 4.88 Hz), 3.86 (4Hs, t, J = 5.12 Hz), 6.83 (2Hs, d, J = 9.52 Hz), 8.14 (2Hs, d, J = 9.52 Hz); ESI-MASS: 231.0 (M+23).

**1-Methyl-4-(4-nitro-phenyl)-piperazine (23b).** To a mixture of 4-fluoro nitro benzene **19b** (425 mg, 3.01 mmol) and K<sub>2</sub>CO<sub>3</sub> (623 mg, 4.52 mmol) in dimethyl sulfoxide (3 mL) was added 1-methyl piperazine (0.66 mL, 6.02 mmol) and the reaction continued as described above to afford 612 mg of amine **23b** in 92% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 2.36 (3Hs, s), 2.55 (4Hs, t, J = 5.12

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Hz), 3.44 (4Hs, t, J = 5.37 Hz), 6.83 (2Hs, d, J = 9.52 Hz), 8.12 (2Hs, d, J = 9.52 Hz); ESI-MASS: 222.1 (M+1).

**1-Benzyl-4-(4-nitro-phenyl)-piperazine (24b).** To a mixture of 4-fluoro nitro benzene **19b** (425 mg, 3.01 mmol) and K<sub>2</sub>CO<sub>3</sub> (623 mg, 4.52 mmol) in dimethyl sulfoxide (3 mL) was added 1-benzyl piperazine (1.04 mL, 6.02 mmol) and the reaction continued as described above to afford amine 805 mg of **24b** in 90% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 2.59 (4Hs, t, J = 4.88 Hz), 3.42 (4Hs, t, J = 5.12 Hz), 3.57 (2Hs, s), 6.81 (2Hs, d, J = 7.32 Hz), 7.29 (1H, sextet, J = 1.22 Hz), 7.34 (4Hs, d, J = 7.39 Hz), 8.12 (2Hs, d; J = 7.32 Hz); ESI-MASS: 298.2 (M+1).

**4-Benzyl-1-(4-nitro-phenyl)-piperidine (25b).** To a mixture of 4-fluoro nitro benzene **19b** (425 mg, 3.01 mmol) and K<sub>2</sub>CO<sub>3</sub> (623 mg, 4.52 mmol) in dimethyl sulfoxide (3 mL) was added 4-benzyl piperidine (1.06 mL, 6.02 mmol) and the reaction continued as described above to afford amine 847 mg of **25b** in 95% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 1.33 (2Hs, dq, J = 3.9, 12.45, 23.92 Hz), 1.74-1.88 (3Hs, m), 2.57 (2Hs, d, J = 6.83 Hz), 2.91 (2Hs, t, J = 15.13 Hz), 3.93 (2Hs, d, J = 13.18 Hz), 6.78 (2Hs, d, J = 9.52 Hz), 7.15 (2Hs, d, J = 7.08 Hz), 7.22 (1H, t, J = 7.32 Hz), 7.30 (2Hs, t, J = 7.56 Hz), 8.1 (2Hs, d, J = 9.52 Hz); ESI-MASS: 319.1 (M+23).

**1-(4-Nitro-phenyl)-4-pyridin-2-yl-piperazine (26b).** To a mixture of 4-fluoro nitro benzene **19b** (425 mg, 3.01 mmol) and K<sub>2</sub>CO<sub>3</sub> (623 mg, 4.52 mmol) in dimethyl sulfoxide (3 mL) was added 1-pyridin-2-yl-piperazine (0.91 mL, 6.02 mmol) and the reaction continued as described above to afford amine 770 mg of **26b** in 90% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 3.6 (4Hs, t, J = 5.12 Hz), 3.76 (4Hs, t, J = 5.61 Hz), 6.65-6.72 (2Hs, m), 6.86 (2Hs, d, J = 9.52 Hz), 7.54 (1H, dt, J = 1.95, 7.07 Hz), 8.16 (2Hs, d, J = 9.5 Hz), 8.22-8.25 (1H, m); ESI-MASS: 285.5 (M+1).

**4-(3-Nitro-phenyl)-morpholine (27b).** To a mixture of 3-fluoro nitro benzene **20b** (425 mg, 3.01 mmol) and K<sub>2</sub>CO<sub>3</sub> (623 mg, 4.52 mmol) in dimethyl sulfoxide (3 mL) was added morpholine (0.52 mL, 6.02 mmol) and the reaction continued as described above to afford amine 589 mg of **27b** in 94% yields. <sup>1</sup>H-

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NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.27 (4Hs, t,  $J$  = 4.83 Hz), 3.9 (4Hs, t,  $J$  = 4.95 Hz), 7.21 (1H, ddd,  $J$  = 1.03, 2.25, 9.06 Hz), 7.42 (1H, t,  $J$  = 8.19 Hz), 7.68-7.76 (2Hs, m); ESI-MASS: 231.0 ( $M+23$ ).

5        **1-Methyl-4-(3-nitro-phenyl)-piperazine (28b)**. To a mixture of 3-fluoro nitro benzene **20b** (425 mg, 3.01 mmol) and  $\text{K}_2\text{CO}_3$  (623 mg, 4.52 mmol) in dimethyl sulfoxide (3 mL) was added 1-methyl piperazine (0.66 mL, 6.02 mmol) and the reaction continued as described above to afford 619 mg of amine **28b** in 93% yields.  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.38 (3Hs, s), 2.6 (4Hs, t,  $J$  = 5.0 Hz), 3.32 (4Hs, t,  $J$  = 5.14 Hz), 7.2 (1H, dd,  $J$  = 2.16, 8.26 Hz), 7.39 (1H, t,  $J$  = 8.14 Hz), 7.67 (1H, dd,  $J$  = 1.43, 8.01 Hz); ESI-MASS: 222.4 ( $M+1$ ).

10        **1-Benzyl-4-(3-nitro-phenyl)-piperazine (29b)**. To a mixture of 3-fluoro nitro benzene **20b** (425 mg, 3.01 mmol) and  $\text{K}_2\text{CO}_3$  (623 mg, 4.52 mmol) in dimethyl sulfoxide (3 mL) was added 1-benzyl piperazine (1.04 mL, 6.02 mmol) and the reaction continued as described above to afford amine 805 mg of **29b** in 90% yields.  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.64 (4Hs, t,  $J$  = 5.0 Hz), 3.31 (4Hs, t,  $J$  = 5.14 Hz), 3.6 (2Hs, s), 7.18 (1H, dd,  $J$  = 1.95, 8.33 Hz), 7.26-7.42 (6Hs, m), 7.66 (1H, ddd,  $J$  = 0.63, 2.02, 8.0 Hz), 7.72 (1H, t,  $J$  = 2.32 Hz); ESI-MASS: 298.1( $M+1$ ).

20        **4-Benzyl-1-(3-nitro-phenyl)-piperidine (30b)**. To a mixture of 3-fluoro nitro benzene **20b** (425 mg, 3.01 mmol) and  $\text{K}_2\text{CO}_3$  (623 mg, 4.52 mmol) in dimethyl sulfoxide (3 mL) was added 4-benzyl piperidine (1.06 mL, 6.02 mmol) and the reaction continued as described above to afford amine 811 mg of **30b** in 91% yields.  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.4 (2Hs, dq,  $J$  = 4.14, 12.82, 24.75 Hz), 1.63-1.88 (3Hs, m), 2.61 (2Hs, d,  $J$  = 6.72 Hz), 2.78 (2Hs, dt,  $J$  = 2.53, 12.5 Hz), 3.77 (2Hs, d,  $J$  = 12.53 Hz), 7.15-7.39 (7Hs, m), 7.62 (1H, dd,  $J$  = 1.59, 7.98 Hz), 7.71 (1H, t,  $J$  = 2.31); ESI-MASS: 319.1 ( $M+23$ ).

25        **1-(3-Nitro-phenyl)-4-pyridin-2-yl-piperazine (31b)**. To a mixture of 3-fluoro nitro benzene **20b** (425 mg, 3.01 mmol) and  $\text{K}_2\text{CO}_3$  (623 mg, 4.52 mmol) in dimethyl sulfoxide (3 mL) was added 1-pyridin-2-yl-piperazine (0.91 mL, 6.02 mmol) and the reaction continued as described above to afford amine 770 mg of



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**31b** in 90% yields.  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.43 (4Hs, t,  $J = 5.41$  Hz), 3.76 (4Hs, t,  $J = 5.38$  Hz), 6.67-6.78 (2Hs, m), 7.24 (1H, ddd,  $J = 0.7, 2.49, 8.33$  Hz), 7.42 (1H, t,  $J = 8.14$  Hz), 7.55 (1H, ddd,  $J = 1.99, 7.16, 8.64$  Hz), 7.7 (1H, ddd,  $J = 0.81, 2.09, 8.04$  Hz), 7.78 (1H, t,  $J = 2.29$  Hz), 8.24 (1H, ddd,  $J = 0.9, 1.95, 4.91$  Hz); ESI-MASS: 285.2 ( $M+1$ ).

**4-Morpholin-4-yl-benzonitrile (44b).** To a mixture of 4-fluorobenzonitrile **42b** (1.0 g, 8.25 mmol) and  $\text{K}_2\text{CO}_3$  (2.27 mg, 16.51 mmol) in dimethyl sulfoxide (7 mL) was added morpholine (1.07 mL, 12.38 mmol) and the reaction continued as described above to afford amine 1.38 g of **44b** in 89% yields.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.32 (4Hs, t,  $J = 4.88$  Hz), 3.89 (4Hs, t,  $J = 4.88$  Hz), 6.91 (2Hs, d,  $J = 8.78$  Hz), 7.54 (2Hs, d,  $J = 8.78$  Hz); ESI-MASS: 189.2 ( $M+1$ ).

**4-(4-Methyl-piperazin-1-yl)-benzonitrile (45b).** To a mixture of 4-fluorobenzonitrile **42b** (1.0 g, 8.25 mmol) and  $\text{K}_2\text{CO}_3$  (2.27 mg, 16.51 mmol) in dimethyl sulfoxide (7 mL) was added 1-methyl piperazine (1.36 mL, 12.38 mmol) and the reaction continued as described above to afford amine 1.54 g of **45b** in 93% yields.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.36 (3Hs, s), 2.55 (4Hs, t,  $J = 4.88$  Hz), 3.35 (4Hs, t,  $J = 4.88$  Hz), 6.87 (2Hs, d,  $J = 8.78$  Hz), 7.49 (2Hs, d,  $J = 8.78$  Hz); ESI-MASS: 202.1 ( $M+1$ ).

**4-(4-Benzyl-piperazin-1-yl)-benzonitrile (46b).** To a mixture of 4-fluorobenzonitrile **42b** (1.0 g, 8.25 mmol) and  $\text{K}_2\text{CO}_3$  (2.27 mg, 16.51 mmol) in dimethyl sulfoxide (7 mL) was added 1-benzyl piperazine (2.14 mL, 12.38 mmol) and the reaction continued as described above to afford amine 2.08 g of **46b** in 91% yields.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.58-2.68 (4Hs, bs), 3.33-3.42 (4Hs, bs), 3.61 (3Hs, s), 6.88 (2Hs, d,  $J = 8.54$  Hz), 7.31-7.44 (5Hs, m), 7.52 (2Hs, d,  $J = 8.54$  Hz); ESI-MASS: 278.2 ( $M+1$ ).

**4-(4-Benzyl-piperidin-1-yl)-benzonitrile (47b).** To a mixture of 4-fluorobenzonitrile **42b** (1.0 g, 8.25 mmol) and  $\text{K}_2\text{CO}_3$  (2.27 mg, 16.51 mmol) in dimethyl sulfoxide (7 mL) was added morpholine (2.17 mL, 12.38 mmol) and the reaction continued as described above to afford amine 2.18 g of **47b** in 96% yields.  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.37 (2Hs, dq,  $J = 3.9, 12.93, 25.14$  Hz),

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1.69-1.84 (3Hs, m), 2.62 (2Hs, d, J = 6.83 Hz), 2.85 (2Hs, dt, J = 2.19, 12.69 Hz), 3.87 (2Hs, d, J = 13.18 Hz), 6.87 (2Hs, d, J = 9.03 Hz), 7.2 (2Hs, d, J = 8.05 Hz), 7.26 (1H, t, J = 7.56 Hz), 7.34 (2Hs, t, J = 7.56 Hz), 7.5 (2Hs, t, J = 9.03 Hz); ESI-MASS: 299.7 (M+1).

5           **3-(4-Methyl-piperazin-1-yl)-benzonitrile (48b).** To a mixture of 3-fluorobenzonitrile **43b** (1.0 g, 8.25 mmol) and K<sub>2</sub>CO<sub>3</sub> (2.27 mg, 16.51 mmol) in dimethyl sulfoxide (7 mL) was added 1-methyl piperazine (1.36 mL, 12.38 mmol) and the reaction continued as described above to afford amine 1.37 g of **48b** in 83% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 2.42 (3Hs, s), 2.63 (4Hs, t, J = 4.88 Hz), 3.29 (4Hs, t, J = 4.88 Hz), 7.13-7.18 (3Hs, m), 7.37 (1H, dd, J = 7.56, 9.27 Hz); ESI-MASS: 202.2 (M+1).

10           **3-(4-Benzyl-piperazin-1-yl)-benzonitrile (49b).** To a mixture of 3-fluorobenzonitrile **43b** (1.0 g, 8.25 mmol) and K<sub>2</sub>CO<sub>3</sub> (2.27 mg, 16.51 mmol) in dimethyl sulfoxide (7 mL) was added 1-benzyl piperazine (2.14 mL, 12.38 mmol) and the reaction continued as described above to afford amine 199 g of **49b** in 87% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 2.65 (4Hs, t, J = 5.12 Hz), 3.27 (4Hs, t, J = 5.12 Hz), 3.61 (2Hs, s), 7.11-7.15 (3Hs, m), 7.31-7.41 (6Hs, m); ESI-MASS: 300.5 (M+23).

15           **3-(4-Benzyl-peperidin-1-yl)-benzonitrile (50b).** To a mixture of 3-fluorobenzonitrile **43b** (1.0 g, 8.25 mmol) and K<sub>2</sub>CO<sub>3</sub> (2.27 mg, 16.51 mmol) in dimethyl sulfoxide (7 mL) was added 1-methyl piperazine (2.17 mL, 12.38 mmol) and the reaction continued as described above to afford amine 2.05 g of **50b** in 90% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 1.43 (2Hs, dq, J = 3.9, 12.45, 23.43 Hz), 1.73-1.86 (3Hs, m), 2.65 (2Hs, d, J = 6.83 Hz), 2.88 (2Hs, dt, J = 2.68, 12.45 Hz), 3.73 (2Hs, d, J = 12.45 Hz), 7.1 (1H, td, J = 0.97, 7.56 Hz), 7.14-7.17 (2Hs, m), 7.22 (2Hs, d, J = 6.83 Hz), 7.27 (1H, t, J = 7.32 Hz), 7.34-7.38 (3Hs, m); ESI-MASS: 299.5 (M+23).

20           **General procedure for preparation of 32b – 39b.** To the substituted nitro compound (1 eq) in mixture of solvents methanol-ethyl acetate (1:2) was added 10% Pd-carbon (5% w/w) and subjected to hydrogenation under 50 Psi hydrogen gas pressure at room temperature. The reaction was monitored by

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TLC, after completion of reaction the reaction mix was filtered thorough celite bed and concentrated in vacuum to afford pure product in quantitative yields.

**4-Morpholin-4-yl-phenylamine (32b).** The 4-(4-Nitro-phenyl)-morpholine **22b**, (600 mg, 2.88 mmol) in a mixture of solvents methanol-ethyl acetate (1:2, 20 mL) was treated with 10% Pd-carbon (5% w/w) under the conditions as described above to afford amine **32b** in quantitative yield. The obtained product was used in the next reaction without further purification and characterization except mass-spectrometric analysis, ESI-MASS: 179.1 (M+1).

**4-(4-Methyl-piperazin-1-yl)-phenylamine (33b).** The 1-Methyl-4-(4-nitro-phenyl)-piperazine **23b**, (600 mg, 2.71 mmol) in mixture of solvents methanol-ethyl acetate (1:2, 20 mL) was treated with 10% Pd-carbon (5% w/w) under the conditions as described above to afford amine **33b** in quantitative yield. <sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD): δ 2.41 (3Hs, s), 2.68 (4Hs, t, J = 4.63 Hz), 3.08-3.13 (4Hs, bs), 3.33 (2Hs, q, J = 1.70, 3.17 Hz), 6.73 (2Hs, d, J = 8.3 Hz), 6.86 (2Hs, d, J = 8.54 Hz); ESI-MASS: 192.2 (M+1).

**4-(4-Benzyl-piperidin-1-yl)-phenylamine (34b).** The 4-Benzyl-1-(4-nitro-phenyl)-piperidine **25b**, (600 mg, 2.02 mmol) in mixture of solvents methanol-ethyl acetate (1:2, 20 mL) was treated with 10% Pd-carbon (5% w/w) under the conditions as described above to afford amine **34b** in quantitative yield. <sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD). δ 1.44 (2Hs, q, J = 9.27, 21.23 Hz), 1.6-1.7 (1H, m), 1.75 (1H, d, J = 12.2 Hz), 2.55 (2Hs, t, J = 11.47 Hz), 2.6 (2Hs, d, J = 7.08 Hz), 3.39 (2Hs, d, J = 11.23. Hz), 6.71 (2Hs, d, J = 7.81 Hz), 6.87 (2Hs, d, J = 8.05 Hz), 7.16-7.21 (3Hs, m), 7.28 (2Hs, t, J = 7.07 Hz); ESI-MASS: 267.1 (M+1).

**4-(4-Pyridin-2-yl-piperazin-1-yl)-phenylamine (35b).** The 1-(4-Nitro-phenyl)-4-pyridin-2-yl-piperazine **26b**, (600 mg, 2.11 mmol) in mixture of solvents methanol-ethyl acetate (1:2, 20 mL) was treated with 10% Pd-carbon (5% w/w) under the conditions as described above to afford amine **35b** in quantitative yield. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 3.14 (4Hs, t, J = 4.88 Hz), 3.68 (4Hs, t, J = 5.12 Hz), 6.62-6.72 (4Hs, m), 6.86 (2Hs, d, J = 8.78 Hz), 7.5 (1H, ddd, J = 2.19, 7.32, 9.03 Hz), 8.21 (1H, dd, J = 1.95, 4.15); ESI-MASS: 255.2 (M+1).

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**3-Morpholin-4-yl-phenylamine (36b).** The 4-(3-Nitro-phenyl)-morpholine **27b**, (600 mg, 2.88 mmol) in mixture of solvents methanol-ethyl acetate (1:2, 20 mL) was treated with 10% Pd-carbon (5% w/w) under the conditions as described above to afford amine **36b** in quantitative yield. <sup>1</sup>H-NMR (300MHz, CDCl<sub>3</sub>): δ 3.145 (4Hs, t, J = 4.79 Hz), 3.86 (4Hs, t, J = 4.89 Hz), 6.24-6.29 (2Hs, m), 6.37 (1H, ddd, J = 0.73, 2.21, 8.20 Hz), 7.08 (1H, t, J = 8.28); ESI-MASS: 201.3 (M+23).

**3-(4-Methyl-piperazin-1-yl)-phenylamine (37b).** The 1-Methyl-4-(3-nitro-phenyl)-piperazine **28b**, (600 mg, 2.71 mmol) in mixture of solvents methanol-ethyl acetate (1:2, 20 mL) was treated with 10% Pd-carbon (5% w/w) under the conditions as described above to afford amine **37b** in quantitative yield. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ 2.36 (3Hs, s), 2.57 (4Hs, t, J = 4.94 Hz), 3.2 (4Hs, t, J = 5.11 Hz), 3.59-3.67 (2Hs, bs), 6.22 (1H, dd, J = 2.07, 8.41 Hz), 6.28 (1H, t, J = 2.21 Hz), 6.39 (1H, dd, J = 1.89, 7.82 Hz), 7.06 (1H, t, J = 8.02 Hz); ESI-MASS: 192.1 (M+1).

**3-(4-Benzyl-piperidin-1-yl)-phenylamine (38b).** The 4-Benzyl-1-(3-nitro-phenyl)-piperidine **30b**, (600 mg, 2.02 mmol) in mixture of solvents methanol-ethyl acetate (1:2, 20 mL) was treated with 10% Pd-carbon (5% w/w) under the conditions as described above to afford amine **38b** in quantitative yield. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 1.4 (2Hs, ddd, J = 4.15, 11.96, 23.92 Hz), 1.61-1.69 (3Hs, m), 2.48-2.64 (4Hs, m), 3.47-3.64 (4Hs, m), 6.18 (1H, dd, J = 1.22, 7.81 Hz), 6.25-6.29 (1H, bs), 6.36 (1H, dd, J = 1.46, 8.3 Hz), 7.02 (1H, dt, J = 1.22, 8.3 Hz), 7.16-7.25 (3Hs, m), 7.29 (2Hs, t, J = 6.59); ESI-MASS: 267.4 (M+1).

**3-(4-Pyridin-2-yl-piperazin-1-yl)-phenylamine (39b).** The 1-(3-Nitro-phenyl)-4-pyridin-2-yl-piperazine **31b**, (600 mg, 2.11 mmol) in mixture of solvents methanol-ethyl acetate (1:2, 20 mL) was treated with 10% Pd-carbon (5% w/w) under the conditions as described above to afford amine **39b** in quantitative yield. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 3.28 (4Hs, dd, J = 3.41, 5.12 Hz), 3.5-3.8 (6Hs, m), 6.26 (1H, ddd, J = 0.73, 1.95, 7.81 Hz), 6.31 (1H, d, J = 2.19 Hz), 6.41 (1H, ddd, J = 0.48, 2.19, 8.05 Hz), 6.65 (1H, ddd, J = 0.73, 4.88, 7.07 Hz), 6.71

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(1H, d, J = 8.54 Hz), 7.07 (1H, dt, J = 1.95, 8.05 Hz), 7.51 (1H, dt, J = 1.95, 7.08 Hz), 8.09-8.22 (1H, m); ESI-MASS: 255.3 (M+1).

**General procedure for preparation of 40b and 41b.** To a solution of the substituted nitro benzene in ethyl acetate (10 mL/mmol)  $\text{SnCl}_2 \cdot \text{H}_2\text{O}$  (1.125 g/mmol) was added. The solution was refluxed for 2 h. The cooled solution was diluted with water and the pH was adjusted to 7-8 by addition of saturated  $\text{NaHCO}_3$  solution. The aqueous phase was extracted with EtOAc (3 x 75 mL) and the combined organic extracts were thoroughly washed with brine and dried over  $\text{MgSO}_4$ . The products obtained after the removal of the solvent were used without further purification.

**4-(4-Benzyl-piperazin-1-yl)-phenylamine (40b).** To a solution of the 1-benzyl-4-(4-nitro-phenyl)-piperazine **24b** (750 mg, 2.52 mmol) in ethyl acetate (30 mL)  $\text{SnCl}_2 \cdot \text{H}_2\text{O}$  (14.4 g, 63.9 mmol) was added. The reaction was carried out as described above to afford 552 mg of amine **40b** in 82% yield.  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.6-2.8 (4Hs, bs), 3.11 (4Hs, t, J = 4.38 Hz), 3.64 (2Hs, s), 6.66 (2Hs, d, J = 8.76 Hz), 6.82 (2Hs, d, J = 8.76 Hz), 7.25-7.45 (5Hs, m); ESI-MASS: 268.2 (M+1).

**3-(4-Benzyl-piperazin-1-yl)-phenylamine (41b).** To a solution of the 1-benzyl-4-(3-nitro-phenyl)-piperazine **29b** (750 mg, 2.52 mmol) in ethyl acetate (30 mL)  $\text{SnCl}_2 \cdot \text{H}_2\text{O}$  (14.4 g, 63.9 mmol) was added. The reaction was carried out as described above to afford 539 mg of amine **41b** in 82% yield.  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.58-2.75 (4Hs, bs), 3.17-3.32 (4Hs, bs), 3.56-3.71 (4Hs, bs), 6.21-6.29 (2Hs, m), 6.37 (1H, dd, J = 1.89, 8.15 Hz), 7.09 (1H, t, J = 4.57 Hz), 7.25-7.47 (5Hs, m); ESI-MASS: 268.3 (M+ 1).

**General procedure for reduction of nitriles 44b – 50b.** To a solution of substituted aryl nitrile (1 mmol) in THF (5 mL) at 0°C was added 65% red-Al in toluene (3 mmol) was added drop wise with stirring under argon atmosphere. The reaction was stirred for 3 h. at room temperature. The reaction was quenched by adding 1 mL methanol drop wise at 0°C followed by 1 mL water. The reaction mixture was filtered through a celite bed washed with THF and the combined fractions were concentrated under vacuum. The resulting crude

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mixture was used in further reaction without further purification and characterization.

**General procedure for preparation of 21b.** 5-Nitro-furan-2-carboxylic acid (942 mg, 6 mmol) in DCM (10 mL) was treated with oxalylchloride (1.04 mL, 12 mmol) followed by 2 drops of DMF and stirred at room temperature for 4 hrs. The reaction mix was concentrated in vacuum to obtain acid chloride and the crude was used in further reactions without purification and characterization.

**General procedure for preparation of compounds 54 – 70.** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of amine (2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was followed by TLC, after completion of reaction 100 mL of ethyl acetate was added and washed with saturated aq. NaHCO<sub>3</sub> (2 x 50 mL), water (2 x 50 mL) and brine (2 x 50 mL). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated followed by flash column purification provided corresponding amides.

**5-Nitro-furan-2-carboxylic acid (4-morpholin-4-yl-phenyl)-amide (54).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of amine 32 (356 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs. at room temperature. Reaction was carried out as explained above to afford 526 mg of amide 54 in 83% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 3.19 (4Hs, t, J = 4.88 Hz), 3.90 (4Hs, t, J = 4.21 Hz), 6.97 (2Hs, d, J = 8.78 Hz), 7.37 (1H, d, J = 3.90 Hz), 7.43 (1H, d, J = 3.90 Hz), 7.67.60 (2Hs, d, J = 8.78 Hz), 8.20 (1H, bs); <sup>13</sup>C-NMR (300 MHz, CDCl<sub>3</sub>): ppm 48.83, 66.27, 12.14, 115.56, 121.24, 128.25, 147.64, 148.53, 153.16; ESI-MASS: 318.8 (M+1).

**5-Nitro-furan-2-carboxylic acid [4-(4-methyl-piperazin-1-yl)-phenyl]-amide (55).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of amine 33b (356 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was carried out as explained above to afford 593 mg of amide 55 in 90% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 2.39 (3Hs, s), 2.62 (4Hs, t, J = 4.63 Hz), 3.25 (4Hs,

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t, J = 5.12 Hz), 6.96 (2Hs, d, J = 9.0 Hz), 7.37 (1H, d, J = 3.90), 7.43 (1H, d, J = 3.90), 7.57 (2Hs, d, J = 9.0 Hz), 8.13 (1H, bs); <sup>13</sup>C-NMR (300 MHz, CDCl<sub>3</sub>): ppm 45.56, 48.52, 54.47, 112.14, 115.81, 121.18, 127.83, 147.68, 148.56, 153.118; ESI-MASS: 331.6 (M+1).

5                   **5-Nitro-furan-2-carboxylic acid [4-(4-benzyl-piperazin-1-yl)-phenyl]-amide (56).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of amine **40b** (534 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was carried out as explained above to afford 755 mg of amide **56** in 93% yields.

10       <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ2.66-2.76 (4Hs, bs), 3.26-3.33 (4Hs, bs), 3.64-3.7 (2Hs, bs), 7.99 (2Hs, d, J = 9.03 Hz), 7.32-7.37 (2Hs, m), 7.3 8-7.46 (4Hs, m), 7.47 (1H, d, J = 3.90), 7.61 (2Hs, d, J = 9.03 Hz), 8.14 (1H, bs); <sup>13</sup>C-NMR (300 MHz, CDCl<sub>3</sub>-DMSO-D<sub>6</sub>, 5:1): ppm 48.13, 51.98, 61.85, 111.94, 114.87, 114.91, 121.36, 126.12, 127.29, 128.12, 128.67, 137.17, 147.65, 148.11, 150.69,

15       153.39; ESI-MASS: 407.5 (M+1). Anal. Calcd. for C<sub>22</sub>H<sub>22</sub>N<sub>4</sub>O<sub>4</sub>: C, 65.01; H, 5.46; N, 13.78. Found: C, 64.94; H, 5.41; N, 13.64.

**5-Nitro-furan-2-carboxylic acid [4-(4-benzyl-piperidin-1-y1)-phenyl]-amide (57).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of amine **34b** (532 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was carried out as explained above to afford 728 mg of amide **57** in 90% yields.

20       <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ1.50-1.65 (3Hs, m), 1.65-1.85 (2Hs, m), 2.6-2.8 (4Hs, m), 3.68 (2Hs, d, J = 10.98 Hz), 6.9-7.02 (2Hs, bs), 7.2-7.3 (3Hs, m), 7.3-7.39 (2Hs, m), 7.41 (1H, d, J = 3.66), 7.47 (1H, t, J = 2.1, 3.6), 7.515-7.65 (2Hs,

25       bs), 8.12-8.22 (1H, bs); <sup>13</sup>C-NMR 300 MHz, (CDCl<sub>3</sub>): ppm 31.37, 37.26, 42.58, 49.36, 112.13, 115.74, 116.16, 121.15, 125.38, 127.34, 127.70, 128.59, 139.84, 147.74, 149.11, 153.06; ESI-MASS:406.4 (M+1).

**5-Nitro-furan-2-carboxylic acid. [4-(4-pyridin-2-yl-piperazin-1-yl)-phenyl]-amide (58).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of amine **35b** (508 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature.

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Reaction was carried out as explained above to afford 723 mg of amide **58** in 92% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 3.37-3.42 (4Hs, bs), 3.75-3.88 (4Hs, bs), 6.54-6.65 (2Hs, bd), 7.02 (2Hs, d, J = 9.03 Hz), 7.38 (1H, d, J = 3.66 Hz), 7.44 (1H, d, J = 3.66 Hz), 7.61 (2Hs, d, J = 9.04 Hz), 8.15 (1H, bs), 8.29 (1H, dd, J = 1.46, 4.88 Hz); <sup>13</sup>C-NMR (300 MHz, CDCl<sub>3</sub>): ppm 44.69, 48.62, 106.69, 112.14, 113.11, 115.85, 116.14, 121.22, 128.16, 137.01, 147.50, 147.64, 148.55, 153.13, 158.85; ESI-MASS: 394.4 (M+1). Anal. Calcd. for C<sub>20</sub>H<sub>19</sub>N<sub>5</sub>O<sub>4</sub>: C, 61.06; H, 4.87; N, 17.80. Found: C, 60.77; H, 4.93; N, 17.57.

**5-Nitro-furan-2-carboxylic acid (3-morpholin-4-yl-phenyl)-amide (59).**

5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of amine **36b** (356 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was carried out as explained above to afford 494 mg of amide **59** in 78% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 3.23 (4Hs, t, J = 4.63 Hz), 3.89 (4Hs, t, J = 4.88 Hz), 6.77 (1H, dd, J = 2.19, 8.3 Hz), 7.1 (1H, dd, J = 1.46, 7.81 Hz), 7.30 (1H, t, J = 8.05 Hz), 7.38 (1H, d, J = 3.66 Hz), 7.46-7.5 (2Hs, m), 8.20 (1H, bs); <sup>13</sup>C-NMR (300 MHz, CDCl<sub>3</sub>): ppm 51.75, 69.37, 110.78, 114.99, 115.26, 115.31, 119.17, 132.16, 140.47, 150.80, 154.44, 157.39; ESI-MASS: 318.3 (M+1).

**5-Nitro-furan-2-carboxylic acid [3-(4-methyl-piperazin-1-yl)-phenyl]-amide (60).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of amine **37b** (382 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was carried out as explained above to afford 593 mg of amide **60** in 90% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 2.38 (3Hs, s), 2.60 (4Hs, t, J = 4.64 Hz), 3.28 (4Hs, t, J = 4.51 Hz), 6.8 (1H, d, J = 8.3 Hz), 7.08 (1H, d, J = 8.05 Hz), 7.25-7.32 (2Hs, m), 7.37-7.45 (2Hs, m), 7.48 (1H, dd, J = 1.22, 3.66 Hz), 8.17 (1H, bs); <sup>13</sup>C-NMR (300 MHz, CD<sub>3</sub>OD): ppm 44.16, 47.92, 53.98, 107.99, 111.48, 111.70, 112.26, 115.49, 128.50, 137.57, 147.55, 151.16, 154.73; ESI-MASS: 331.3 (M+1). Anal. Calcd. for C<sub>16</sub>H<sub>18</sub>N<sub>4</sub>O<sub>4</sub>: C, 58.17; H, 5.49; N, 16.96. Found: C, 57.94; H, 5.40; N, 16.92.



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**5-Nitro-furan-2-carboxylic acid [3-(4-benzyl-piperazin-1-yl)-phenyl]-amide (61).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of amine **41b** (534 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was carried out as explained above to afford 747 mg of amide **61** in 92% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 2.6-2.74 (4Hs, bs), 3.26-3.36 (4Hs, bs), 3.59-3.7 (2Hs, bs), 6.76 (1H, dd, J = 2.19, 8.05 Hz), 7.09 (1H, d, J = 8.05 Hz), 7.25-7.35 (3Hs, m), 7.35-7.42 (4Hs, m), 7.43 (1H, d, J = 3.66), 8.04 (1H, s), 8.2 15 (1H, s); <sup>13</sup>C-NMR (300 MHz, CDCl<sub>3</sub>): ppm 48.23, 52.41, 62.46, 107.31, 110.79, 112.16, 112.25, 116.02, 126.60, 127.73, 128.63, 129.09, 136.98, 137.38, 147.68, 150.72, 151.50, 153.52; ESI-MASS: 407.5 (M+1).

**5-Nitro-furan-2-carboxylic acid [3-(4-benzyl-piperidin-1-yl)-phenyl]-amide (62).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of amine **38b** (532 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was carried out as explained above to afford 777 mg of amide **62** in 96% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 1.50-1.67 (3Hs, m), 1.67-1.88 (2Hs, m), 2.63 (2Hs, d, J = 6.84), 2.73-2.88 (2Hs, m), 3.78 (2Hs, d, J = 12.20 Hz), 6.78-6.84 (1H, bs), 7.04-7.16 (1H, bs), 7.16-7.36 (8Hs, m), 7.38 (1H, d, J = 3.9 Hz), 7.43 (1H, d, J = 7 Hz), 8.15-8.28 (1H, bs); <sup>13</sup>C-NMR (300 MHz, CDCl<sub>3</sub>): ppm 31.30, 37.31, 42.58, 48.92, 76.70, 107.60, 110.38, 112.23, 112.70, 116.0, 125.40, 127.73, 128.63, 129.05, 136.96, 139.89, 147.66, 150.74, 151.81, 153.63; ESI-MASS: 406.4 (M+1).

**5-Nitro-furan-2-carboxylic acid [3-(4-pyridin-2-yl-piperazin-1-yl)-phenyl]-amide (63).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of amine **39b** (508 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was carried out as explained above to afford 682 mg of amide **63** in 85% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 3.40 (4Hs, t, J = 5.37 Hz), 3.72-3.82 (4Hs, bs), 6.71 (1H, t, J = 5.85 Hz), 6.76 (1H, d, J = 8.54), 6.83 (1H, dd, J = 2.44, 8.05 Hz), 7.11 (1H, dd, J = 1.7, 7.56 Hz), 7.32 (1H, t, J = 8.05 Hz), 7.4 (1H, d, J =

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3.90Hz), 7.44 (1H, d, J = 3.66), 7.47 (1H, t, J = 2.19 Hz), 7.56-7.62 (1H, bs), 8.29 (1H, s), 8.25 (1H, dd, J = 1.70, 4.88 Hz); <sup>13</sup>C-NMR (300 MHz, CDCl<sub>3</sub>): ppm 44.47, 48.02, 107.21, 107.85, 111.55, 112.13, 113.18, 113.38, 116.29, 129.17, 137.48, 138.54, 147.51, 148.01, 151.27, 151.64, 154.42, 158.90; ESI-MASS: 394.4 (M+1). Anal. Calcd. for C<sub>20</sub>H<sub>19</sub>N<sub>5</sub>O<sub>4</sub>: C, 61.06; H, 4.87; N, 17.80. Found: C, 61.09; H, 4.95; N, 17.60.

**5-Nitro-furan-2-carboxylic acid 4-morpholin-4-yl-benzylamide (64).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of crude amine **44b** (384 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was carried out as explained above to afford 469 mg of amide **64** in 71% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 3.22 (4Hs, t, J = 4.88 Hz), 3.92 (4Hs, t, J = 4.88 Hz), 4.61 (2Hs, d, J = 5.61 Hz), 6.78-6.82 (1H, bs), 6.96 (2Hs, d, J = 8.78 Hz), 7.3 1-7.35 (3Hs, m), 7.41 (1H, d, J = 3.90 Hz); <sup>13</sup>C-NMR (300 MHz, DMSO-D<sub>6</sub>): ppm 41.81, 48.56, 65.99, 113.34, 114.99, 115.42, 128.39, 129.20, 148.29, 150.23, 151.37, 155.88; ESI-MASS: 332.4 (M+1); Anal. Calcd. for C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>5</sub>: C, 58.00; H, 5.17; N, 12.68. Found: C, 57.71; H, 5.23; N, 12.41.

**5-Nitro-furan-2-carboxylic acid 4-(4-methyl-piperazin-1-yl)-benzylamide (65).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of crude amine **45b** (410 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was carried out as explained above to afford 481 mg of amide **65** in 70% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 2.41 (3Hs, s), 2.63 (4Hs, t, J = 4.88 Hz), 3.27 (4Hs, t, J = 4.88 Hz), 4.6 (2Hs, d, J = 5.61 Hz), 6.78-6.83 (1H, bs), 6.97 (2Hs, d, J = 8.78 Hz), 7.31 (2Hs, d, J = 8.78 Hz), 7.33 (1H, d, J = 3.90 Hz), 7.41 (1H, d, J = 3.90 Hz); <sup>13</sup>C-NMR (300 MHz, CDCl<sub>3</sub>): ppm 41.71, 44.02, 48.06, 53.94, 111.31, 114.88, 115.52, 127.89, 128.91, 147.54, 149.95, 156.61; ESI-MASS: 345.3 (M+1); Anal. Calcd. for C<sub>17</sub>H<sub>2</sub>ON<sub>4</sub>O<sub>4</sub>: C, 59.29; H, 5.85; N, 16.27. Found: C, 59.16; H, 5.91; N, 16.19.

**5-Nitro-furan-2-carboxylic acid 4-(4-benzyl-piperazin-1-yl)-benzylamide (66).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in

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CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of crude amine **46b** (562 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was carried out as explained above to afford 363 mg of amide **66** in 79% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 2.66 (4Hs, t, J = 5.12 Hz), 3.26 (4Hs, t, J = 5.12 Hz), 3.62 (3Hs, s), 4.60 (2Hs, d, J = 5.61 Hz), 6.77-6.82 (1H, bs), 6.96 (2Hs, d, J = 8.78 Hz), 7.30 (2Hs, d, J = 8.54 Hz), 7.31-7.35 (4Hs, m), 7.37-7.43 (4Hs, m); <sup>13</sup>C-NMR (300 MHz, CDCl<sub>3</sub>): ppm 42.67, 48.40, 52.44, 62.50, 111.84, 115.39, 115.53, 126.62, 126.94, 127.74, 128.63, 128.65, 137.39, 147.60, 150.65, 155.46; ESI-MASS: 421.5 (M+1).

**5-Nitro-furan-2-carboxylic acid 4-(4-benzyl-piperidin-1-yl)-benzylamide (67).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of crude amine **47b** (560 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was carried out as explained above to afford 720 mg of amide **67** in 86% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 1.46 (2Hs, dq, J = 3.9, 12.44, 23.92 Hz), 1.69-1.83 (3Hs, m), 2.64 (2Hs, d, J = 7.08 Hz), 2.72 (2Hs, dt, J = 2.44, 12.2 Hz), 3.72 (2Hs, d, J = 12.45 Hz), 4.58 (2Hs, d, J = 5.85 Hz), 6.78-6.82 (1H, bs), 6.96 (2Hs, d, J = 8.78 Hz), 7.2-7.3 (5Hs, m), 7.32 (1H, d, J = 4.0 Hz), 7.35 (2Hs, t, J = 7.32 Hz), 7.40 (1H, d, J = 3.66 Hz); <sup>13</sup>C-NMR (300 MHz, CDCl<sub>3</sub>): ppm 31.35, 37.33, 42.60, 42.70, 49.19, 111.88, 115.38, 115.93, 125.39, 126.53, 127.71, 128.49, 128.60, 128.62, 139.86, 147.65, 150.98, 155.48; ESI-MASS: 420.6 (M+1).

**5-Nitro-furan-2-carboxylic acid 3-(4-methyl-piperazin-1-yl)-benzylamide (68).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of crude amine **48b** (410 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was carried out as explained above to afford 474 mg of amide **68** in 69% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 2.41 (3Hs, s), 2.64 (4Hs, t, J = 4.88 Hz), 3.29 (4Hs, t, J = 4.88 Hz), 4.63 (2Hs, d, J = 5.85 Hz), 6.89 (2Hs, d, J = 7.32), 6.93-6.98 (2Hs, m), 7.3-7.36 (2Hs, m), 7.42 (1H, d, J = 3.66 Hz); ESI-MASS: 345.1 (M+ 1).

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**5-Nitro-furan-2-carboxylic acid 3-(4-benzyl-piperazin-1-yl)-benzylamide (69).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of crude amine **49b** (562 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was carried out as explained above to afford 604 mg of amide **69** in 72% yields. <sup>1</sup>H-NMR. (500 MHz, CDCl<sub>3</sub>): δ 2.66 (4Hs, t, J = 4.88 Hz), 3.272 (4Hs, t, J = 5.12 Hz), 3.62 (2Hs, s), 4.63 (2Hs, d, J = 5.85 Hz), 6.87 (1H, d, J = 7.56 Hz), 6.9-6.95 (3Hs, m), 7.29-7.35 (3Hs, m), 3.7-3.5 (5Hs, m); ESI-MASS: 421.5 (M+1).

**5-Nitro-furan-2-carboxylic acid 3-(4-benzyl-piperidin-1-yl)-benzylamide (70).** 5-Nitro-furan-2-carbonyl chloride (438 mg, 2.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to a mixture of crude amine **50b** (560 mg, 2.0 mmol) in Et<sub>3</sub>N (1.04 mL, 7.5 mmol) and the mixture was stirred for 12 hrs at room temperature. Reaction was carried out as explained above to afford 695 mg of amide **70** in 83% yields. <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ 1.44 (2Hs, dq, J = 3.66, 11.71, 23.92 Hz), 1.68-1.77 (1H, m), 1.78 (2Hs, d, J = 13.18 Hz), 2.62 (2Hs, d, J = 2.83 Hz), 2.7 (2Hs, dt, J = 2.19, 12.20 Hz), 3.72 (2Hs, d, J = 12.45 Hz), 4.61 (2Hs, d, J = 5.61 Hz), 6.81-6.88 (2Hs, m), 6.9-6.94 (2Hs, m), 7.21 (2Hs, d, J = 7.07 Hz), 7.22-7.36 (5Hs, m), 7.40 (1H, d, J = 3.66 Hz); <sup>13</sup>C-NMR (300 MHz, CDCl<sub>3</sub>): ppm 31.41, 37.30, 42.57, 43.54, 49.17, 111.84, 115.27, 115.48, 118.09, 125.28, 127.70, 128.59, 129.10, 137.20, 139.85, 147.52, 151.74, 155.52; ESI-MASS: 420.4 (M+1); Anal. Calcd. for C<sub>24</sub>H<sub>25</sub>N<sub>3</sub>O<sub>4</sub>: C, 68.72, H, 6.01; N, 10.02. Found: C, 68.33; H, 6.01; N, 10.02.

### Biological Results

The MIC of the nitrofuranyl amides against *M tuberculosis* H37Ra was determined by the micro broth dilution method using microtiter plates. *M. tuberculosis* was grown in Middlebrook 7H9 medium to an OD<sub>650</sub> of 0.4 - 0.6 and a dilution made to an. OD<sub>650</sub> of 0.01. 100 μl of these cells are then added to a microtiter well containing serial dilutions of the nitrofuranyl amides. The cells are then incubated at 37°C for 7 days and visually examined for growth. MIC<sub>90</sub> was

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determined for wells with greater than 90% inhibition of growth. Results are shown in Tables 4 and 5 below.

5 The synthesis of cyclic, tertiary amine substituted, benzyl nitrofuranyl amides required the preparation of amine ring building blocks, which were synthesized by nucleophilic displacement of nitro or cyano phenyl fluorides with the selected secondary amine, followed by reduction of the cyano or nitro group to produce the desired benzylamine and phenylamines respectively. The remaining amides targeted in this series of compounds were synthesized by acylation of the corresponding amine using our standard procedure from  
10 Example 1. Thus, a set of: 8 benzyl nitrofuranyl amides; 3 fused tertiary benzyl nitrofuranyl amides; 18 cyclic tertiary amine substituted benzyl and phenyl nitrofuranyl amides; and 19 benzyl or phenyl piperaziny l nitrofuranyl amides was designed and synthesized.

15 The choice of substitution in this series was based on the first series of compounds in Example 1 and on the developing knowledge of the SAR of the series. Some of the most active second generation compounds in this series are shown in Tables 4 and 5. More interesting SAR was developed in this series: (i) constrained tertiary amides, such as compound **71**, have increased activity over N-methyl aniline amide **41** (Example 1; MIC 3.1  $\mu$ g/ml) (ii) benzyl piperazine **73**, and piperidine amide **72**, had good activity compared to N-methyl piperazine **76**  
20 (MIC 37.5  $\mu$ g/ml) (iii) tertiary amide **75**, an analog of the *in vivo* active compound **23**, was very potent (iv) increasing the solubility of the benzyl nitrofuranyl amide series by substituting the benzyl ring with a cyclic amine, such as compound **66**, also increased the potency against *M. tuberculosis*, suggesting that in addition to  
25 increasing the solubility, these substitutions may also affect the bacterial uptake.

Example 2 describes the synthesis of the target molecules in good yields. As can be seen by the data, no barrier to scale up synthesis for larger quantities for *in vivo* testing is offered by these synthesis schemes. Therefore, *in vivo* testing using the techniques disclosed herein, along with general knowledge and  
30 skills presently available in the art can be readily achieved by one of ordinary skill in the art.

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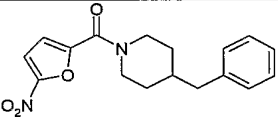
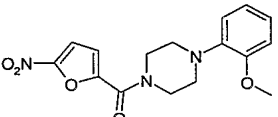
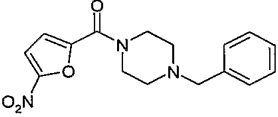
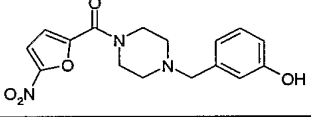
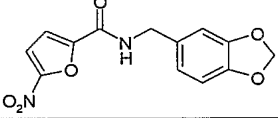
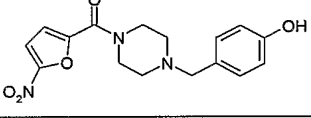
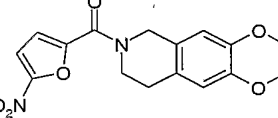
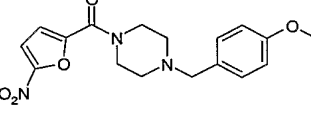
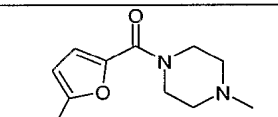
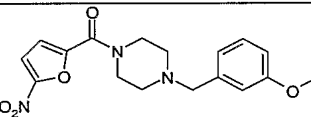
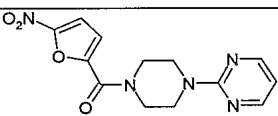
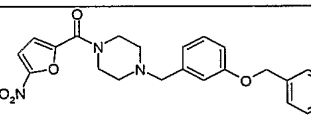
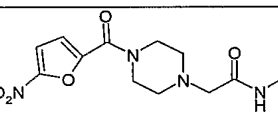
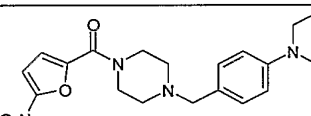
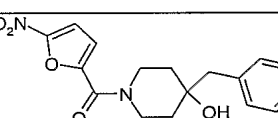
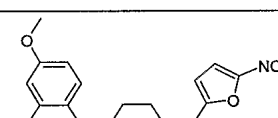
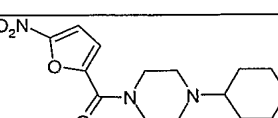
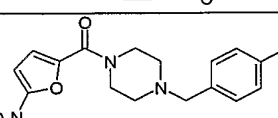
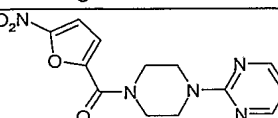
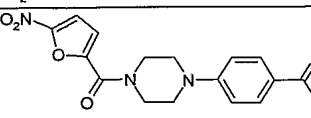
5        There is a clear structure activity relationship for the compounds described in Example 2, with the substituted benzyl compounds having greater anti-tuberculosis than the substituted phenyl compounds (see Table 4 below). In both the phenyl and the benzyl amides *para*-substitution with the cyclic secondary amine produced better anti-tuberculosis activity. Compounds **66** and **70**, both from the benzyl series, are extremely potent and are the most active compounds so far developed in this class.

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Table 4 Cyclic secondary amine substituted phenyl and benzyl nitrofuranyl amides and their anti-tuberculosis activity					
No.	Compound	MIC ( $\mu\text{g/mL}$ )	No.	Compound	MIC ( $\mu\text{g/mL}$ )
54		1.6	63		1.6
55		6.25	64		0.1
56		0.8	65		0.1
57		3.1	66		0.0062
58		0.4-0.8	67		<0.1
59		3.1	68		3.1
60		12.5	69		<0.1
61		12.5	70		0.0062
62		12.5	71		0.78

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Table 5  
Additional nitrofuranyl amides and their anti-tuberculosis activity

No.	Compound	MIC ( $\mu\text{g/mL}$ )	No.	Compound	MIC ( $\mu\text{g/mL}$ )
72		0.29	82		1.56
73		0.59	83		0.8
74		0.15	84		1.56
75		0.15	85		<0.1
76		37.5	86		1.56
77		0.025	87		1.56
78		6.25	88		<0.1
79		1.56	89		<0.1
80		6.25	90		-
81		0.1	91		<0.1



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**Comparison of Example 2 lead compounds with other antimycobacterial drugs.**

Two nitrofuranyl amides, one from each round of optimization, were assayed for MIC activity against *M. tuberculosis* H37Ra, along with nitrofurantoin (NFT) and 4 classical TB drugs: ethambutol (EMB), isoniazid (INH), Rifampin (RMP), and streptomycin sulfate (SM) (Figure 1). The MIC values for the TB drugs and nitrofurantoin are similar to those in the literature for *M. tuberculosis* (R.E. Lee et al., *J. Combinatorial Chem.* 2003, 5(2), 172-87). Against H37Ra, compound **23** is as active as ethambutol and streptomycin, and compound **66** is more active than isoniazid, and perhaps even as active as rifampin. It appears that compound **66** has a sub MIC growth impairment effect, although in the virulent strain, H37Rv, the effect is much less pronounced. The MIC activities of these novel compounds in the ng/ml range are very encouraging.

Discussion of Examples 1 and 2

There are structural differences between the nitrofuranyl amides disclosed herein, and particularly in Examples 1 and 2 and the nitrofuranyl imine antibiotics such as nitrofurantoin: (i) the amide bond is more electron withdrawing than the imine linkage, which will alter the reactivity of the nitrofuran group to metabolic activation; (ii) the amide and imine linkages will be metabolized differently, and the introduction of the amide bond can introduce a desirable site for secondary metabolism; (iii) nitrofuranyl imine antibiotics are bicyclic and the nitrofuranyl amides are mostly tricyclic, including one unsaturated ring; and (iv) the most active compounds in the nitrofuranyl amide series are 10,000-100,000-fold more active against *M. tuberculosis* than any of the clinically prescribed nitrofuranylimine antibiotics, suggesting non-bioequivalence.

In summary, Examples 1 and 2 discuss two rounds of lead optimization based on *in vitro* testing that produced potent compounds (see Figure 2). From the discovery of the first screening hit, an optimization library of 43 compounds was synthesized (Example 1), from which compound **23** (MIC 0.2mg/mL and selectivity index (SI) 90.9) was selected as a lead compound for further

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optimization. A second-generation optimization library was synthesized (Example 2), following previously successful antimicrobial design strategies to improve the bioavailability and solubility of the series. These changes also increased the activity against *M. tuberculosis* yielding second-generation lead compound **66** (MIC 6ng/ml, SI 1597). Compounds in this generation were more soluble and better formulated.

The compounds disclosed herein can be further optimized based on *in vivo* testing and toxicology data to achieve increased bioavailability and *in vivo* activity. The metabolism and mode of action of these and further compounds can also be studied, as is known in the art. Importantly, this compound series has a number of promising features that makes them attractive new anti-tuberculosis agents: they possess extremely potent MIC values; they have good selectivity indexes; activity has been demonstrated in an *in vivo* model of tuberculosis infection; the MIC activity of this series is comparable to front-line anti-tuberculosis agents such as isoniazid and ethambutol; the compounds are not cross-resistant with other clinically used anti-tuberculosis agents; the compounds can be easily synthesized at low cost; and lastly, the compounds are novel.

### Example 3

Examples 1 and 2 describe developing compounds with potent anti-tuberculosis activity, with at least 7 compounds with MIC values in the 5-100 ng/mL range. This Example pertains to developing a third generation of compounds and focuses on improving the solubility and bioavailability of the series. Without wishing to be limited by theory, limited bioavailability can be a result of 3 factors: (i) the metabolic instability of the amide; (ii) the solubility of compounds in this class; (iii) high serum binding and poor tissue distribution.

To address the first issue, a number of tertiary amides can be tested and alternative linkages which should have increased stability to proteolysis can be explored. Increasing the solubility of compounds in this series was addressed in Example 2 above by adding an ionizable or polar side chain in the form of a

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substituted piperazine or morpholine rings, a strategy that has been successfully used to develop oral bioavailability in other antimicrobial agents. This strategy led to the successful representative second generation compound **66**, discussed above. As this strategy was clearly successful, continued expansion of the series based on compound **66** is discussed in this example, along with careful monitoring of changes in SAR, and how they influence the predicted and observed bioavailability. Modifying the leads to alter serum binding and tissue distribution can be addressed by testing functional group substitutions that are known to decrease protein binding and through further analysis of the results discussed above.

#### Scheme 1

In the second generation optimization library discussed in Example 2, 18 cyclic tertiary amine substituted at 3 and 4 positions of benzyl (8 compounds) and phenyl (10 compounds) nitrofuranyl amides were synthesized. As the 4-benzyl series showed significantly superior activity, especially compound **66**, this series is further elaborated upon, as shown in Scheme 1 below. The previous compounds were synthesized starting from 3 or 4-fluorobenzonitrile, which was reacted with 4 different substituted piperazines in a nucleophilic aromatic displacement reaction. The nitriles were then reduced and acylated to afford the target compounds in good yields. The previous series is expanded through two major synthesis routes (Scheme 1). Route 1 uses a convenient commercially available starting material that contains both B and C rings and allows for substitution of the piperazine ring prior to reduction of the nitrile. The piperazine ring can be further elaborated by reductive amination using a wide range of commercially available aldehydes and especially important for the developing SAR substituted benzaldehydes. Substitutions to the piperazine ring by alkylation with a variety alkylhalides such as bromomethylcyclopropane can be further explored. Both these elaborations are ideal chemistries for parallel synthesis and should offer no significant synthetic challenges.

Route 2 utilizes nucleophilic aromatic displacement to introduce new B and C rings. Substitutions to the benzyl B ring can be carried out using other

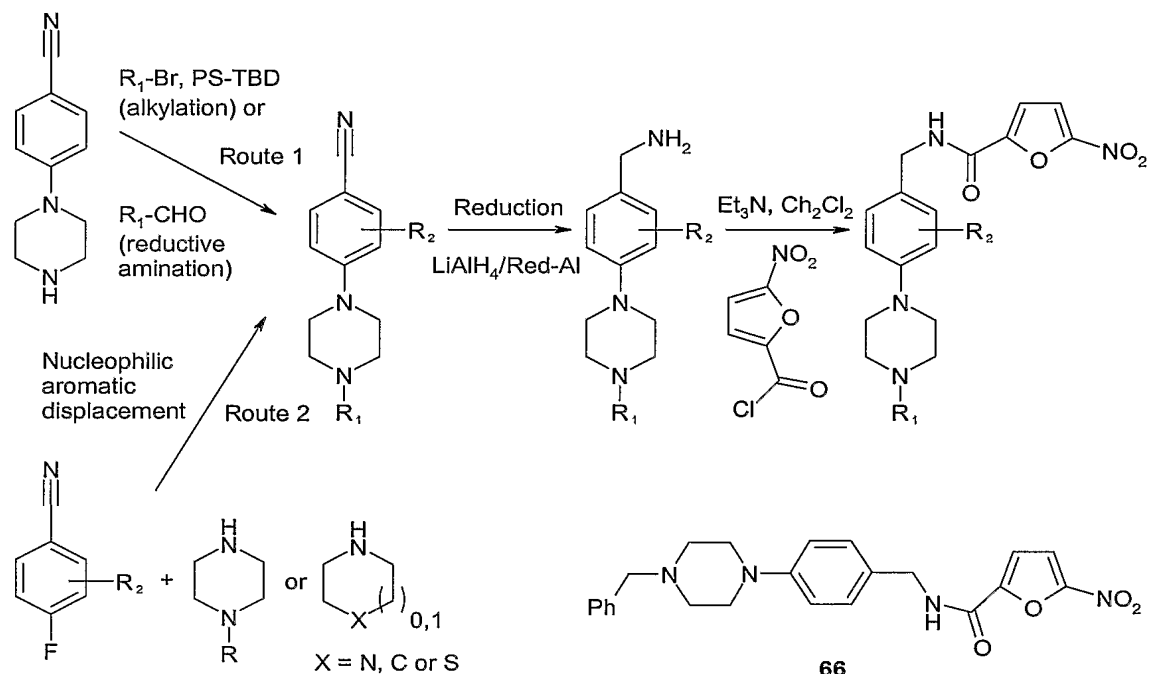
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commercially available trisubstituted 4-fluorobenzonitriles such as 3,4-difluorobenzonitrile. As the 4-position is the most activated the previously used conditions can be applied to synthesize substituted 4-piperazinyl benzyl amides and to evaluate the effects of having a halide substituted benzyl B ring. A number of other cyclic amine substitutions can be tested using novel building blocks to study their effects on the bioavailability of this series. There are a large number of potential cyclic amines commercially available for purchase from commercial entities as is known to one of skill in the art, such as, for example, Maybridge, Maybridge, United Kingdom and Sigma-Aldrich, St. Louis, Missouri, USA.

One area of consideration that factors into the third generation design is the high predicted protein binding values for compound **66** and therefore it is desirable to evaluate compounds with lower protein binding numbers. Thus, each proposed compound can be modeled before starting synthesis to ensure that it has appropriate drug like physical properties. One compound which can be targeted for the third generation series is the thiomorpholine analog, which upon completion of synthesis to the nitrofuranyl amide, can be further oxidized to the corresponding cyclic sulfone, a substitution that typically decreases plasma binding.

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Scheme 1  
Expansion of Compound 66 Series

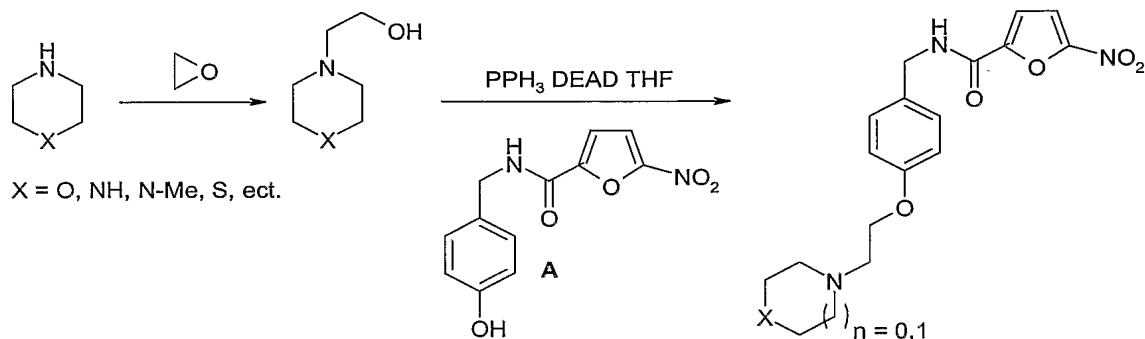


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Scheme 2

Scheme 2 shows synthesis of ether substituted nitrofuranyl benzylamides. To complement the third generation optimization library detailed above the synthesis and activity of substituted phenether nitrofuranyl benzylamides is examined (Scheme 2). The SAR of the 1st generation nitrofuranyl amides (Example 1) indicated that 4-methoxy was the best substitution for bioactivity. N-(4-hydroxybenzyl)-5-nitrofuranyl-2-carboxamide A was synthesized for use as a starting material to synthesize a sub library of 15 compounds by etherification to the corresponding 2-hydroxyethyl cyclic amines, such as 1-(2-hydroxyethyl)piperidine using Mitsunobu chemistry. See Grese et al., *J. Med. Chem.* 2001, 44(17), 2857-2860. Some 1-(2-hydroxy ethyl) piperidines are commercially available, and other desirable 2-hydroxyethyl cyclic amines can easily be generated by the reaction of a cyclic secondary amine and ethylene oxide.

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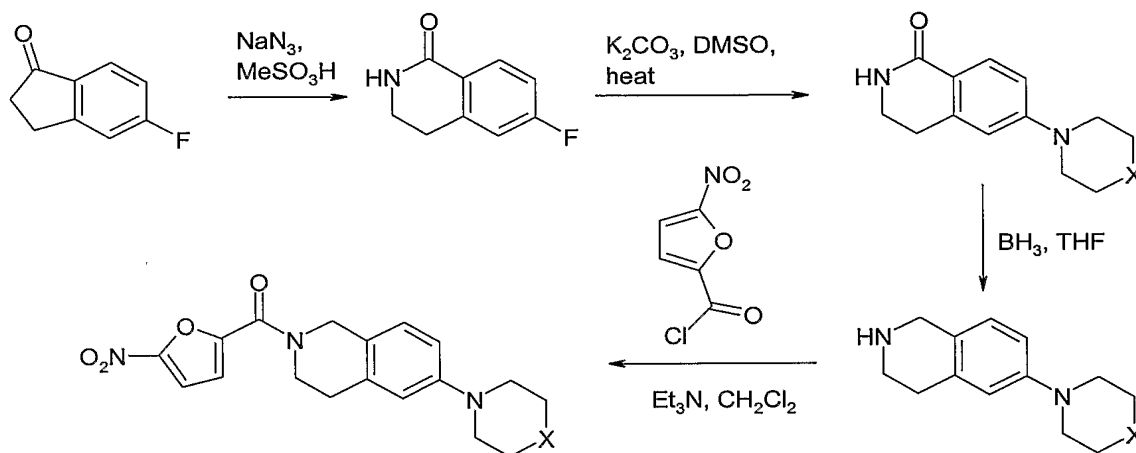
Scheme 2Synthesis of Substituted Ether Nitrofuranyl BenzylamidesScheme 3

5                Scheme 3 describes synthesis of tertiary nitrofuranylamides with piperazinyl substitutions. The third series to expand is the addition of a piperazine ring to the tertiary amide system of compound **75**, to determine if these molecules have enhanced metabolic stabilities over the analogous compounds in the compound **66** series. For the synthesis of this series, 5-fluoroindanone is converted to 6-fluoro-3,4-dihydro-2*H*-isoquinolin-1-one intermediate, which is the Schmidt rearrangement product, as disclosed in PCT

10 Published Application No. WO 2001057039, incorporated herein in its entirety. The isoquinolinone is subjected to nucleophilic substitution with secondary amines followed by reduction of the amide functionality with BH<sub>3</sub>THF to provide

15 the corresponding secondary amine, which can be acylated to give the desired products.

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Scheme 3Synthesis of Tertiary Nitrofuranylamides with Piperazinyl Substitutions

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Examination of alternative heterocycles to nitrofuran

Alternative nitroheterocyclic rings, in addition to nitrofuran, is examined, as an area of SAR optimization. The nitro group plays a role in activity. As such, ten additional nitroheterocyclic ring systems for consideration as anti-tuberculosis compounds are shown in Figure 3.

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A representative panel of 10 amides for each nitroheterocycle is synthesized using the same 10 amines in all cases, for example. The amines are selected as representative active amines in the nitrofuran series and the anti-tuberculosis SAR is determined for the heterocyclic ring. Upon observation of activity, an expanded set of amides is synthesized for each heterocycle and

15

evaluated in an optimization program.

Nitroheterocycles thiofuran II, pyrrole VI, thiazole VIII and pyrazole IX and X series can be synthesized from their corresponding commercially available carboxylic acids using standard methods as is generally known in the art. The synthesis of nitropyrrole I and nitroimidazole IV amides is well established for the synthesis of DNA binding polyamides. For example, the Dervan procedure can be used (Baird & Dervan, *J. Am. Chem. Soc.* 1996, 118, 6141-6146). The thiophene series V can be synthesized by starting from thiophene-2-carboxylic acid, which on nitration gives 5-nitro-thiophene-2-carboxylic acid. See Fu et al.,

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*Amer. Chem. Soc.* 1999, 121(34), 7761-7759. Further amine coupling affords the desired amides. In order to synthesis series VII, 5-nitro-isoxazole-3-carboxylic acid ethyl ester can be prepared according to the procedure of Diamantini et al. (Giuseppe et al., *Synthesis*, 1993, 11, 1104-1108), and further hydrolysis to the acid followed by amine coupling afford the desired final amides.

#### Scheme 4

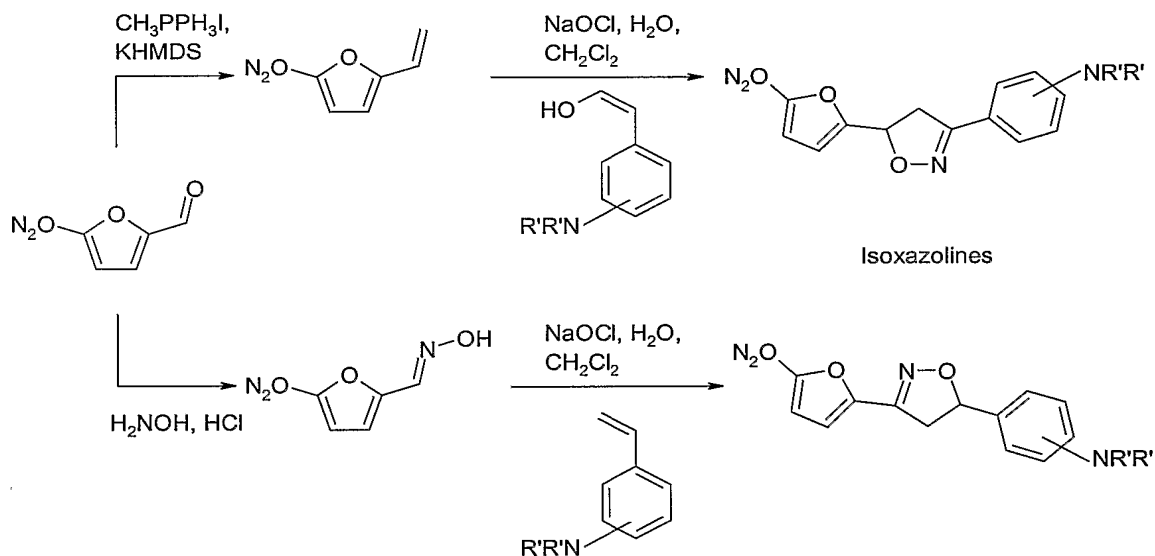
Referring now to Scheme 4, to complete the SAR of the nitrofuranyl amide series the importance of the amide bond is examined, in that alternative linkages can have improved bioavailability. Imine analogs are generated by condensing 5-nitro-2-furaldehyde with the same 10 amines selected in the section immediately proceeding. The vinyl analogs are generated using standard Wittig Chemistry.

Additionally, more advanced and constrained cyclic bioisosteric bridges such as benzoxazole, isoxazoline and isoxazole are investigated in evaluating bioavailability. A benzoxazole bridge is formed by condensation of the amide to C-2 aromatic hydroxyl of 2-aminophenol. Isoxazolines and isoxazoles are synthesized from aryl aldehydes, which are converted to oximes reaction with by simple hydroxylamine hydrochloride. See Barbachyn et al., *J. Med. Chem.* 2003, 46(2), 284-302; and Choi et al., *J. Bacteriol.* 2001, 183, 7058-7066. A nitrile oxide generated from corresponding oximes on reaction with olefins gives isoxazolines. Similarly, isoxazoles can be prepared by reacting alkynes instead of olefins with nitrile oxide. The orientation of furan and aryl groups over the oxazole/oxazoline ring can be directed by exchanging oxime-yne/ene functionality between those two groups.



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**Scheme 4**  
**Isioxazole Synthesis**



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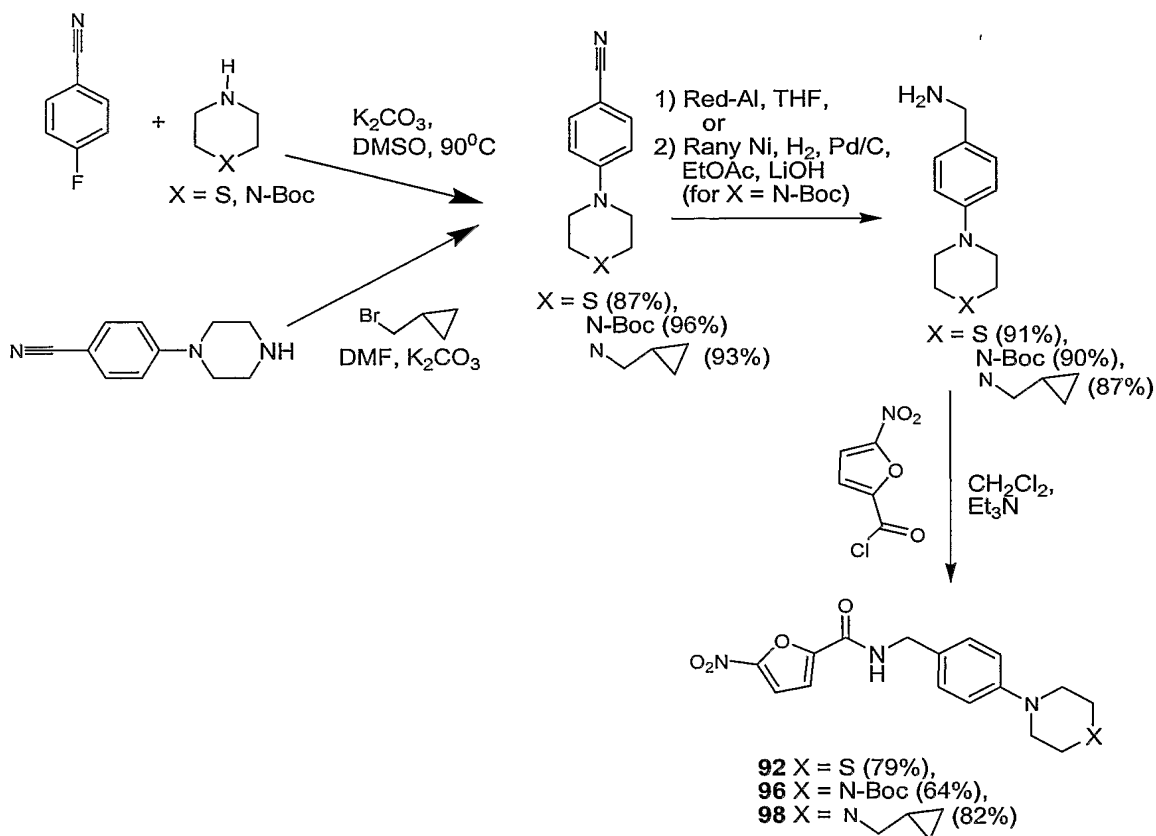
**Example 4****Scheme 1**

Referring now to Scheme 1, the compounds **92** and **96** were synthesized starting from p-fluorobenzonitrile. The fluoro group was substituted with the corresponding cyclic secondary amines. Then the nitrile functionality was reduced with red-Al in case of thiomorpholine substituted benzonitrile and with Raney-Ni hydrogenation in case of N-Boc piperazine substituted benzonitrile to give the corresponding primary amines, which were then treated with 5-nitro-furan-2-carbonyl chloride to give the final product compounds **92** and **96**. The synthesis of compound **98** was carried out in similar way to compound **92** starting from 4-(4-cyclopropylmethyl-piperazin-1-yl)-benzonitrile.

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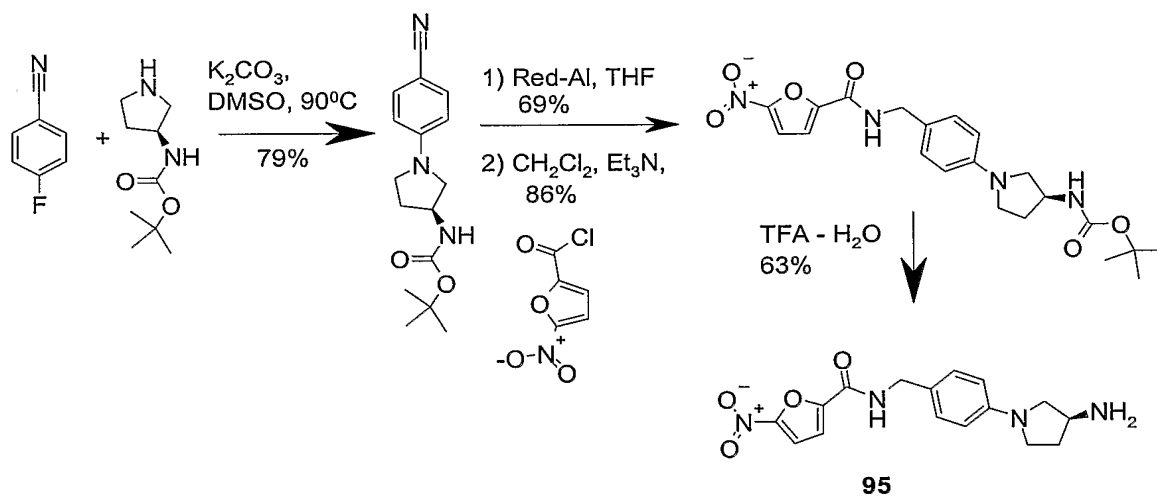
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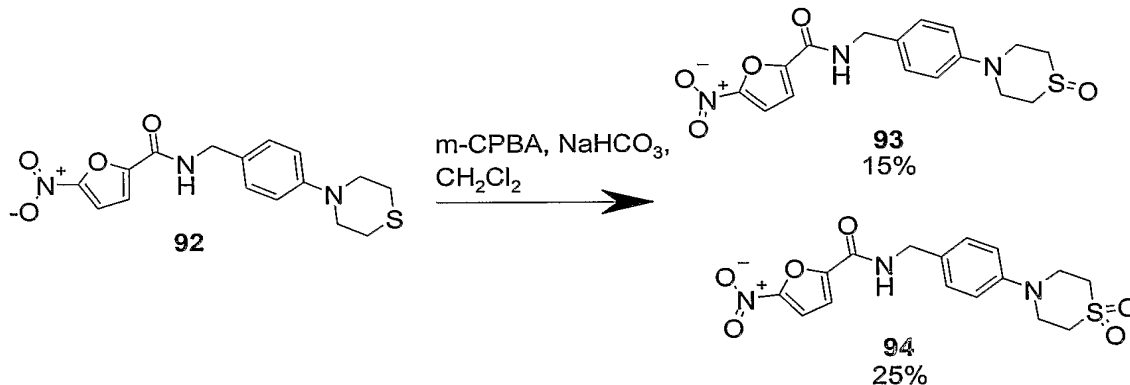
**Scheme 2**

Referring now to Scheme 2, substitution of fluorine on 4-fluorobenzonitrile with (S)-(-)-3-(Boc-amino) pyrrolidine followed by reduction of nitrile group with red-Al gave the corresponding benzylamine. The benzylamine was treated with 5-nitro-furan-2-carbonyl chloride to give the intermediate with on Boc-deprotection gave the product compound **95**.

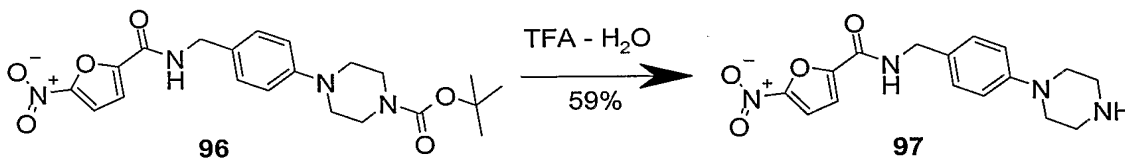
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Scheme 3

Referring now to Scheme 3, compound **92** was further treated with *m*-chloroperbenzoic acid to give oxidized product compounds **93** and **94**.

Scheme 4

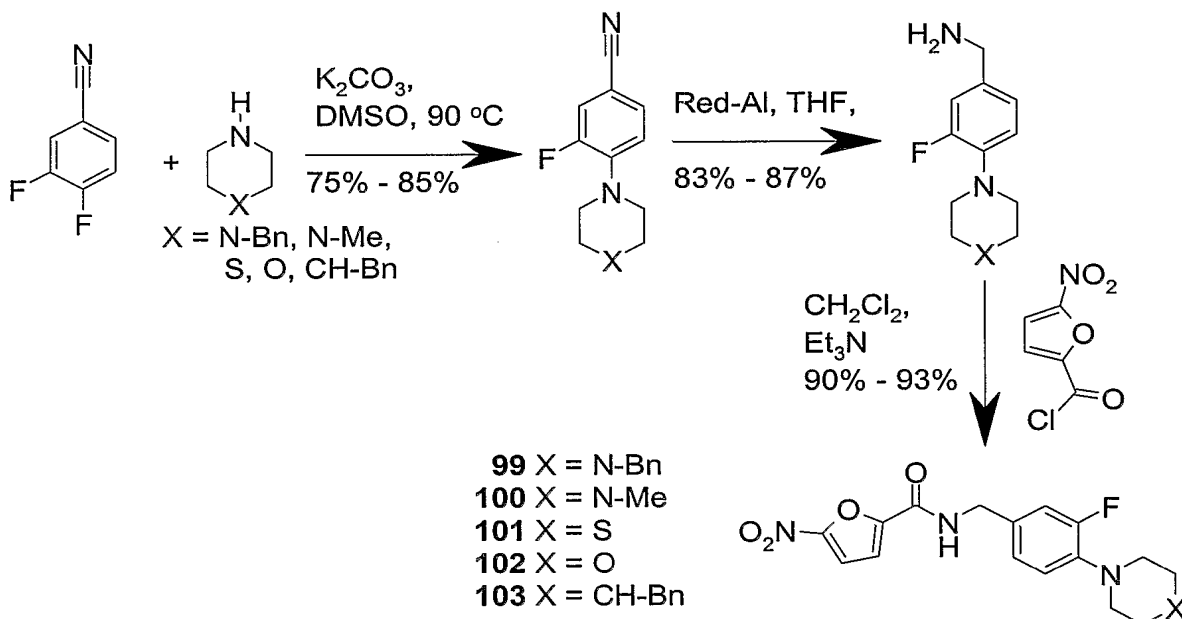
Referring now to Scheme 4, the boc-protecting group on compound **96** was removed by treating with trifluoroacetic acid in water to give free amine compound **97**.

Scheme 5

Referring now to Scheme 5, the targeted products were synthesized starting from 3,4-difluorobenzonitrile, which on reaction with cyclic secondary

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amides in presence of base substituted the *para*-fluorine group and gave the corresponding tertiary amines. Subsequently the nitrile group was reduced with red-Al to give corresponding benzylamines, which were then treated with 5-nitro-furan-2-carbonyl chloride to give the final targeted product compounds **99** - **103**.

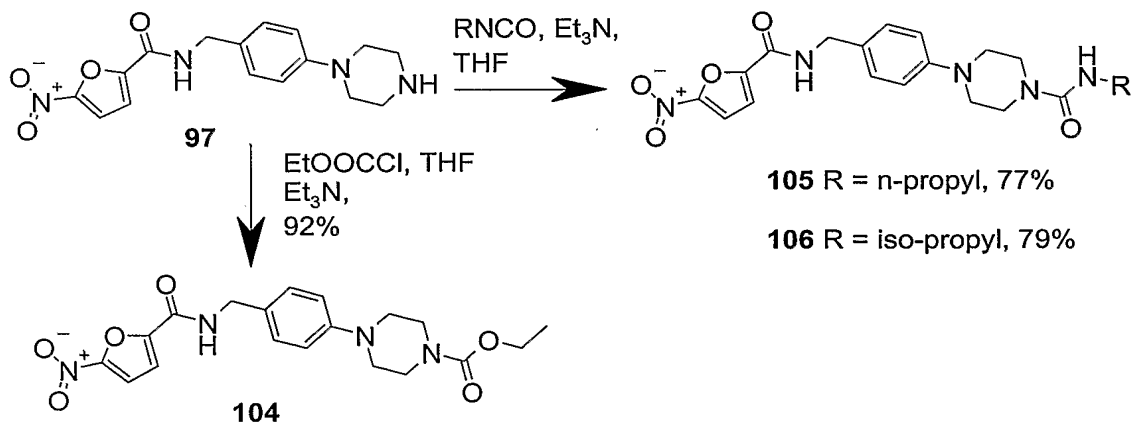


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Scheme 6

Referring now to Scheme 6, compound **97** was treated with ethylchloroformate in presence of triethylamine to give the carbamate compound **104**. Similarly, compound **97** on reaction with *n*-propyl isocyanate and isopropyl isocyanate gave the urea derivatives compounds **105** and **106**, respectively.

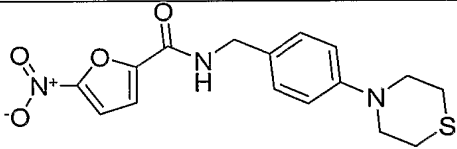
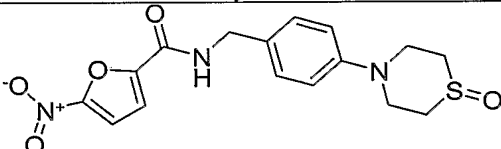
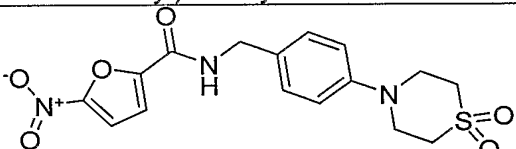
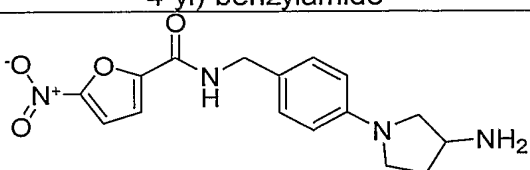
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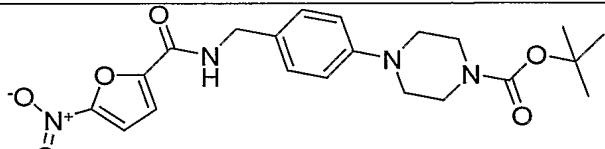
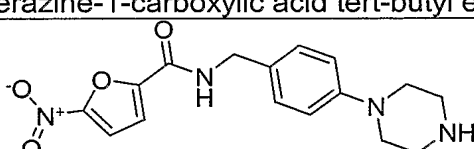
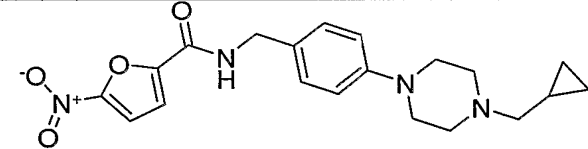
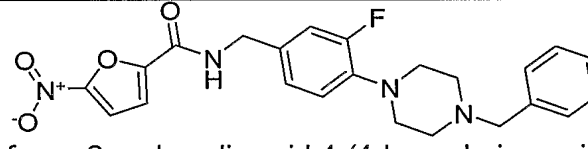
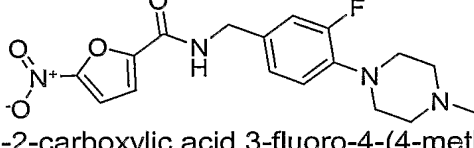
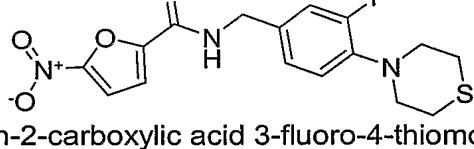
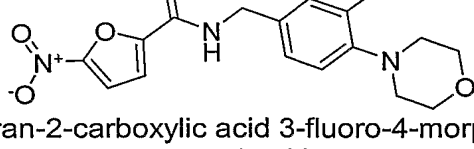
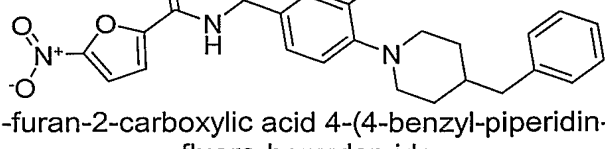
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Biological Results

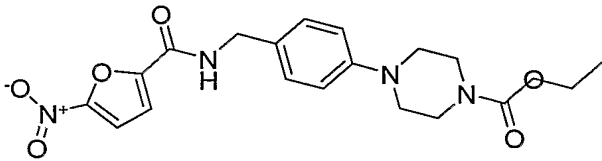
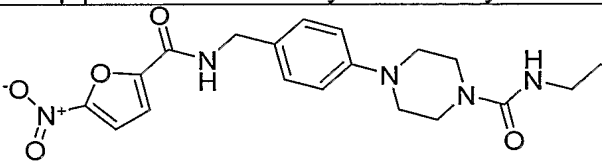
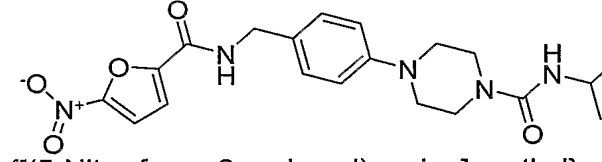
The MIC of the nitrofuranyl amides against *M tuberculosis* H37Ra was determined by the micro broth dilution method using microtiter plates. *M. tuberculosis* was grown in Middlebrook 7H9 medium to an OD<sub>650</sub> of 0.4 - 0.6 and a dilution made to an. OD<sub>650</sub> of 0.01. 100  $\mu$ l of these cells are then added to a microtiter well containing serial dilutions of the nitrofuranyl amides. The cells are then incubated at 37°C for 7 days and visually examined for growth. MIC<sub>90</sub> was determined for wells with greater than 90% inhibition of growth. Results are shown in Table 6 below.

Table 6		
No.	Compound	MIC ( $\mu$ g/ml)
92	 5-Nitro-furan-2-carboxylic acid 4-thiomorpholin-4-yl-benzylamide	0.05
93	 5-Nitro-furan-2-carboxylic acid 4-(1-oxo-1,4-thiomorpholin-4-yl)-benzylamide	0.8
94	 5-Nitro-furan-2-carboxylic acid 4-(1,1-dioxo-1,6-thiomorpholin-4-yl)-benzylamide	6.25
95	 5-Nitro-furan-2-carboxylic acid 4-(3-amino-pyrrolidin-1-yl)-benzylamide	0.8

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96	 <p>4-(4-((5-Nitro-furan-2-carbonyl)-amino)-methyl)-phenyl)-piperazine-1-carboxylic acid tert-butyl ester</p>	0.0002
97	 <p>5-Nitro-furan-2-carboxylic acid 4-piperazin-1-yl-benzylamide</p>	0.8
98	 <p>5-Nitro-furan-2-carboxylic acid 4-(4-cyclopropylmethyl)-piperazin-1-yl)-benzylamide</p>	0.05
99	 <p>5-Nitro-furan-2-carboxylic acid 4-(4-benzyl-piperazin-1-yl)-3-fluoro-benzylamide</p>	0.0125
100	 <p>5-Nitro-furan-2-carboxylic acid 3-fluoro-4-(4-methyl-piperazin-1-yl)-benzylamide</p>	0.4
101	 <p>5-Nitro-furan-2-carboxylic acid 3-fluoro-4-thiomorpholin-4-yl)-benzylamide</p>	0.2
102	 <p>5-Nitro-furan-2-carboxylic acid 3-fluoro-4-morpholin-4-yl)-benzylamide</p>	0.2
103	 <p>5-Nitro-furan-2-carboxylic acid 4-(4-benzyl-piperidin-1-yl)-3-fluoro-benzylamide</p>	0.0125

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104	 4-(4-[[[(5-Nitro-furan-2-carbonyl)-amino]-methyl]-phenyl]-piperazine-1-carboxylic acid ethyl ester	0.004
105	 4-(4-[[[(5-Nitro-furan-2-carbonyl)-amino]-methyl]-phenyl]-piperazine-1-carboxylic acid propylamide	0.0125
106	 4-(4-[[[(5-Nitro-furan-2-carbonyl)-amino]-methyl]-phenyl]-piperazine-1-carboxylic acid isopropylamide	0.0062

It will be understood that various details of the presently disclosed subject matter can be changed without departing from the scope of the presently disclosed subject matter. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.

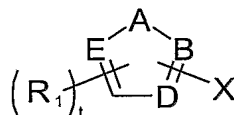
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## CLAIMS

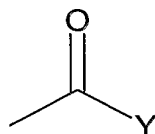
What is claimed is:

1. A compound having the general formula:



- 5 wherein A is selected from the group consisting of oxygen, sulfur, and NR<sub>15</sub>, and R<sub>15</sub> is selected from the group consisting of H, alkyl, aryl, substituted alkyl, and substituted aryl;  
B, D, and E are each independently selected from the group consisting of CH, nitrogen, sulfur and oxygen;
- 10 R<sub>1</sub> is selected from the group consisting of nitro, halo, alkyl ester, phenylsulfanyl, phenylsulfinyl, phenylsulfonyl and sulfonic acid;  
t is an integer from 1 to 3; and  
X is a substituted amide.

- 15 2. The compound of Claim 1, wherein R<sub>1</sub> is nitro.
3. The compound of Claim 1, wherein X has the formula:



and Y is a substituted amine.

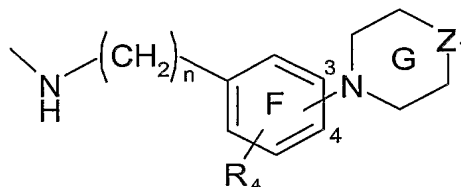
- 20 4. The compound of Claim 3, wherein Y is an aromatic monoamine.
5. The compound of Claim 3, wherein Y has the formula selected from the group consisting of:
- 25 (a) -NR<sub>2</sub>R<sub>3</sub>, and R<sub>2</sub> and R<sub>3</sub> are each independently selected from the group consisting of H, alkyl, aryl, substituted alkyl, and substituted



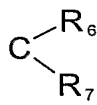
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aryl, or  $R_2$  and  $R_3$  together form a ring structure with the nitrogen atom to which they are attached;

(b)

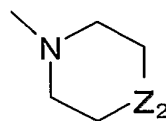


5 wherein  $n$  is an integer from 0 to 8;  $R_4$  is selected from the group consisting of hydrogen, halo, alkyl, substituted alkyl, and alkoxy; and  $Z_1$  is selected from the group consisting of oxygen, sulfur, sulfoxide,

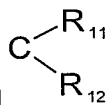


10 sulfone,  $NR_5$ , and  $-(C=O)-NR_8R_9$ ; wherein  $R_5$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkoxycarbonyl, aryl, substituted aryl, and  $-(C=O)-NR_8R_9$ , wherein  $R_6$  and  $R_7$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl and  $R_8$  and  $R_9$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl;

(c)



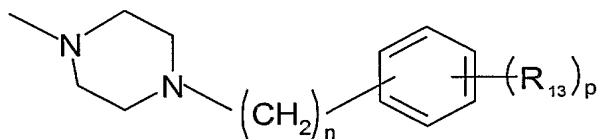
wherein  $Z_2$  is selected from the group consisting of oxygen,  $NR_{10}$ ,



20 and  $-(C=O)-NR_{10}$ ;  $R_{10}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl; and  $R_{11}$  and  $R_{12}$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl;

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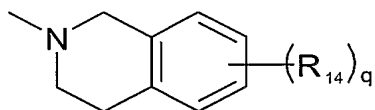
(d)



wherein n is an integer from 0 to 8; p is an integer from 1 to 5; and R<sub>13</sub> is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl;

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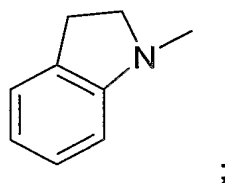
(e)



wherein q is an integer from 1 to 4; and R<sub>14</sub> is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl;

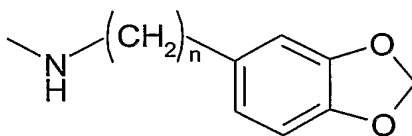
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(f)



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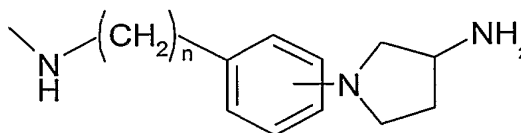
(g)



wherein n is an integer from 0 to 8; and

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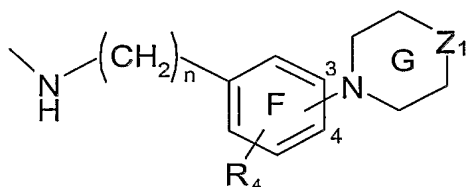
(h)



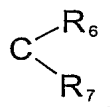
wherein n is an integer from 0 to 8.

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6. The compound of Claim 5, wherein Y is  $\text{NR}_2\text{R}_3$  and wherein  $\text{R}_1$  is nitro,  $\text{R}_2$  is H and  $\text{R}_3$  is aryl or substituted aryl.
7. The compound of Claim 5, wherein Y is  $\text{NR}_2\text{R}_3$  and wherein  $\text{R}_1$  is nitro, and  $\text{R}_2$  and  $\text{R}_3$  together form a ring structure with the nitrogen atom to which they are attached.
8. The compound of Claim 5, wherein Y is

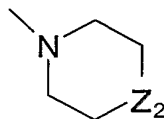


9. The compound of Claim 8 wherein n is zero.
10. The compound of Claim 8, wherein n is one.
11. The compound of Claim 8, wherein ring G is in the 3-position of ring F.
12. The compound of Claim 8, wherein ring G is in the 4-position of ring F.
13. The compound of Claim 8, wherein  $\text{Z}_1$  is oxygen or sulfur.
14. The compound of Claim 8, wherein  $\text{Z}_1$  comprises  $\text{NR}_5$ .
15. The compound of Claim 8, wherein  $\text{Z}_1$  comprises



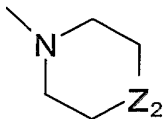
16. The compound of Claim 5, wherein Y is

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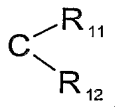


and  $Z_2$  comprises  $NR_{10}$ .

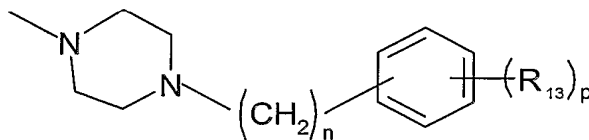
17. The compound of Claim 5, wherein Y is



and  $Z_2$  comprises

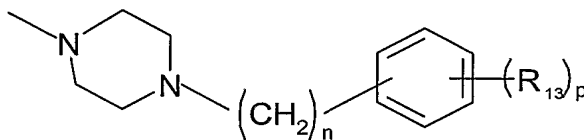


18. The compound of Claim 5, wherein Y is



and n is zero.

19. The compound of Claim 5, wherein Y is



and n is one.

20. The compound of Claim 3, wherein Y is selected from the group consisting of anisidine, 3-halo-aniline, 3-anisidine, 4-anisidine, cyclohexylamine, adamantyl amine, furfuryl amine, 4-amino-benzonitrile, 4-methoxy-benzylamine, 2-chloro-benzylamine, 2,4-dimethoxy-

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benzylamine, 3,4-dimethoxy-benzylamine, 3,4,5-trimethoxy-benzylamine,  
1-amino-1,2,3,4-tetrahydro-napththalene, 1-amino-indane,  
phenethylamine, 4-methoxy-phenethylamine, (S)-1-phenyl-ethylamine,  
(R)-1-phenyl-ethylamine, 3,4-dimethoxy-phenethylamine, 4-methoxy-  
5 benzylamine, 3-amino-phenol, 3-benzyloxy-aniline, N-methyl-aniline, N-  
methyl-4-anisidine, 2,3-dihydro-indole, 2-amino-pyridine, 3-amino-  
pyridine, 4-amino-pyridine, 3-amino-pyrazole, 2-amino-pyrazine, 2-amino  
methyl pyridine, 2-amino-4-methoxy-benzothiazole, 4-amino-6-methoxy-  
pyrimidine, 2-methoxy-benzylamine, and 2,3-dimethoxy-benzylamine.

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21. The compound of Claim 3, wherein Y is 3-chloro-aniline.

22. The compound of Claim 3, wherein Y is 3-fluoro-aniline.

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23. The compound of Claim 3, wherein Y is 3-anisidine.

24. The compound of Claim 3, wherein Y is 4-methoxy-benzylamine.

25. The compound of Claim 3, wherein Y is 3,4-dimethoxy-benzylamine.

20

26. The compound of Claim 8, wherein R<sub>1</sub> is nitro.

27. The compound of Claim 1, wherein the compound is selected from the  
group consisting of 5-nitrofur-2-carboxylic acid (3-chloro-phenyl)-amide;  
25 5-nitrofur-2-carboxylic acid (3-bromo-phenyl)-amide; 5-nitrofur-2-  
carboxylic acid (3-fluoro-phenyl)-amide; 5-nitrofur-2-carboxylic acid (4-  
methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-methoxy-  
phenyl)-amide; 5-nitrofur-2-carboxylic acid adamantan-1-ylamide; 5-  
nitrofur-2-carboxylic acid phenylamide; 5-nitrofur-2-carboxylic acid  
30 (furan-2-ylmethyl)-amide; 5-nitrofur-2-carboxylic acid (4-cyano-phenyl)-  
amide; 5-nitrofur-2-carboxylic acid 4-methoxy-benzylamide; 5-

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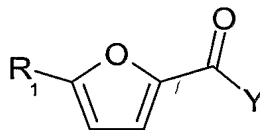
nitrofuran-2-carboxylic acid 2-chlorobenzylamide; 5-nitrofuran-2-carboxylic acid 2,4-dimethoxybenzylamide; 5-nitrofuran-2-carboxylic acid 3,4-dimethoxybenzylamide; 5-nitrofuran-2-carboxylic acid 3,4,5-trimethoxybenzylamide; 5-nitrofuran-2-carboxylic acid (1,2,3,4-tetrahydronaphthalen-1-yl)-amide; 5-nitrofuran-2-carboxylic acid indan-1-ylamide; 5-nitrofuran-2-carboxylic acid phenethyl-amide; 5-nitrofuran-2-carboxylic acid [2-(4-methoxy-phenyl)-ethyl]-amide; 5-nitrofuran-2-carboxylic acid (1-phenyl-ethyl)-amide; 5-nitrofuran-2-carboxylic acid [2-(3,4-dimethoxy-phenyl)-ethyl]-amide; 5-nitrofuran-2-carboxylic acid 4-methoxybenzylamide; 5-nitrofuran-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-sulfo-furan-2-carboxylic acid; 5-(3-methoxy-phenylcarbamoyl)-furan-sulfonic acid; 5-nitrofuran-2-carboxylic acid (3-hydroxy-phenyl)-amide; 5-phenylsulfanyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-nitrofuran-2-carboxylic acid (3-benzyloxy-phenyl)-amide; 5-benzenesulfinyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-benzenesulfonyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-nitrofuran-2-carboxylic acid pyrazin-2-ylamide; 5-nitrofuran-2-carboxylic acid (pyridin-2-yl methyl)-amide; 5-nitrofuran-2-carboxylic acid (4-methoxy-benzothiazol-2-yl)-amide; 5-nitrofuran-2-carboxylic acid (6-methoxy-pyrimin-4-yl)-amide; 5-nitrofuran-2-carboxylic acid 2-methoxybenzylamide; 5-nitrofuran-2-carboxylic acid 2,3-dimethoxybenzylamide; 5-nitrofuran-2-carboxylic acid 4-thiomorpholin-4-yl-benzylamide; 5-nitrofuran-2-carboxylic acid 4-(1-oxo-1,4-thiomorpholin-4-yl)-benzylamide; 5-nitrofuran-2-carboxylic acid 4-(1,1-dioxo-1,4-thiomorpholin-4-yl)-benzylamide; 5-nitrofuran-2-carboxylic acid 4-(3-amino-pyrrolidin-1-yl)-benzylamide; 4-(4-[(5-nitrofuran-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid tert-butyl ester; 5-nitrofuran-2-carboxylic acid 4-piperazin-1-yl-benzylamide; 5-nitrofuran-2-carboxylic acid 4-(4-cyclopropylmethyl-piperazin-1-yl)-benzylamide; 5-nitrofuran-2-carboxylic acid 4-(4-benzyl-piperazin-1-yl)-3-fluorobenzylamide; 5-nitrofuran-2-carboxylic acid 3-fluoro-4-(4-methyl-

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5 piperazin-1-yl)-benzylamide; 5-nitrofuran-2-carboxylic acid 3-fluoro-4-thiomorpholin-4-yl-benzylamide; 5-nitrofuran-2-carboxylic acid 3-fluoro-4-morpholin-4-yl-benzylamide; 5-nitrofuran-2-carboxylic acid 4-(4-benzylpiperidin-1-yl)-3-fluoro-benzylamide; 4-(4-[(5-nitrofuran-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid ethyl ester; 4-(4-[(5-nitrofuran-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid propylamide; and 4-(4-[(5-nitrofuran-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid isopropylamide.

10 28. The compound of Claim 1, wherein the compound is selected from the group consisting of *N*-(4-methoxybenzyl)-5-nitrofuran-2-carboxamide; (3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl)(5-nitrofuran-2-yl)methanone; *N*-((benzo[*d*][1,3]dioxol-6-yl)methyl)-5-nitrofuran-2-carboxamide; *N*-(2,4-dimethoxybenzyl)-5-nitrofuran-2-carboxamide; *N*-(3,4-dimethoxyphenethyl)-5-nitrofuran-2-carboxamide; *N*-(3,4-dimethoxybenzyl)-5-nitrofuran-2-carboxamide; and *N*-(4-methoxyphenyl)-5-nitrofuran-2-carboxamide.

20 29. A method of killing or inhibiting the growth of a microorganism comprising contacting the microorganism with an effective amount of the compound having the general formula:



25 wherein  $R_1$  is selected from the group consisting of halo, nitro, alkyl ester, phenylsulfanyl, phenylsulfinyl, phenylsulfonyl and sulfonic acid; and Y is a substituted amine.

30. The method of Claim 29, wherein the microorganism is a member of the genus *Mycobacterium*.

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31. The method of Claim 30, wherein the microorganism is *Mycobacterium tuberculosis*.

5 32. The method of Claim 29, wherein the effective amount is about 1 to about 1000 micromoles per milliliter of the compound.

33. The method of Claim 32, wherein the effective amount is about 10 to 500 micromoles per milliliter.

10 34. The method of Claim 33, wherein the effective amount is about 50 to 250 micromoles per milliliter.

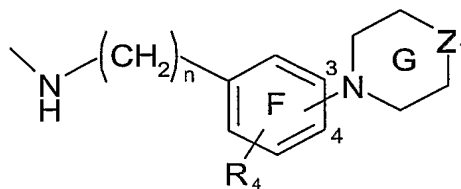
35. The method of Claim 34, wherein the effective amount is about 100 to 200 micromoles per milliliter.

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36. The method of Claim 29, wherein Y is selected from the group consisting of:

20 (c)  $-NR_2R_3$ , and  $R_2$  and  $R_3$  are each independently selected from the group consisting of H, alkyl, aryl, substituted alkyl, and substituted aryl, or  $R_2$  and  $R_3$  together form a ring structure with the nitrogen atom to which they are attached;

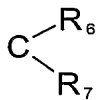
(d)



25 wherein  $n$  is an integer from 0 to 8;  $R_4$  is selected from the group consisting of hydrogen, halo, alkyl, substituted alkyl, and alkoxy; and  $Z_1$  is selected from the group consisting of oxygen, sulfur, sulfoxide,

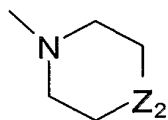


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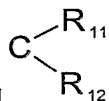


sulfone,  $\text{NR}_5$ , and  $\text{C}(\text{R}_6)(\text{R}_7)$ ; wherein  $\text{R}_5$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkoxy carbonyl, aryl, substituted aryl, and  $-(\text{C}=\text{O})-\text{NR}_8\text{R}_9$ , wherein  $\text{R}_6$  and  $\text{R}_7$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl and  $\text{R}_8$  and  $\text{R}_9$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl;

(c)

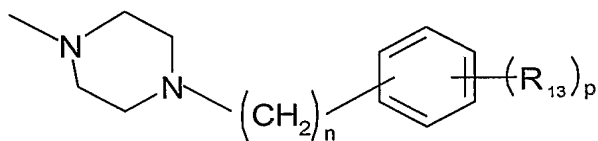


wherein  $\text{Z}_2$  is selected from the group consisting of oxygen,  $\text{NR}_{10}$ ,



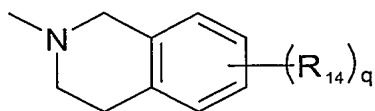
and  $\text{C}(\text{R}_{11})(\text{R}_{12})$ ;  $\text{R}_{10}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl; and  $\text{R}_{11}$  and  $\text{R}_{12}$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl;

(d)



wherein  $n$  is an integer from 0 to 8;  $p$  is an integer from 1 to 5; and  $\text{R}_{13}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl;

(e)

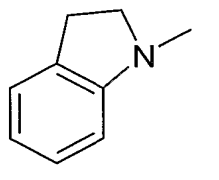


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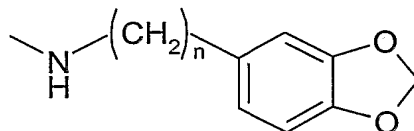
wherein q is an integer from 1 to 4; and R<sub>14</sub> is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl;

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(f)



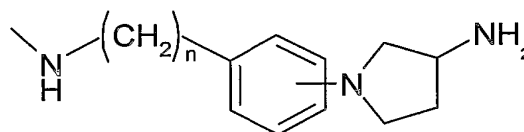
(g)



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wherein n is an integer from 0 to 8; and

(h)



wherein n is an integer from 0 to 8.

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37. The method of Claim 29, wherein Y is selected from the group consisting of anisidine, 3-halo-aniline, 3-anisidine, 4-anisidine, cyclohexylamine, adamantyl amine, furfuryl amine, 4-amino-benzonitrile, 4-methoxy-benzylamine, 2-chloro-benzylamine, 2,4-dimethoxy-benzylamine, 3,4-dimethoxy-benzylamine, 3,4,5-trimethoxy-benzylamine, 1-amino-1,2,3,4-tetrahydro-naphthalene, 1-amino-indane, phenethylamine, 4-ethoxy-phenethylamine, (S)-1-phenyl-ethylamine, (R)-1-phenyl-ethylamine, 3,4-dimethoxy-phenethylamine, 4-methoxy-benzylamine, 3-amino-phenol, 3-

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benzyloxy-aniline, N-methyl-aniline, N-methyl-4-anisidine, 2,3-dihydro-indole, 2-amino-pyridine, 3-amino-pyridine, 4-amino-pyridine, 3-amino-pyrazole, 2-amino-pyrazine, 2-amino methyl pyridine, 2-amino-4-methoxy-benzothiazole, 4-amino-6-methoxy-pyrimidine, 2-methoxy-benzylamine, and 2,3-dimethoxy-benzylamine.

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38. The method of Claim 37, wherein Y is 3-chloro-aniline.

39. The method of Claim 37, wherein Y is 3-fluoro-aniline.

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40. The method of Claim 37, wherein Y is 3-anisidine.

41. The method of Claim 37, wherein Y is 4-methoxy-benzylamine.

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42. The method of Claim 37, wherein Y is 3,4-dimethoxy-benzylamine.

43. The method of Claim 29, wherein the compound is selected from the group consisting of 5-nitrofur-2-carboxylic acid (3-chloro-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-bromo-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-fluoro-phenyl)-amide; 5-nitrofur-2-carboxylic acid (4-methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid adamantan-1-ylamide; 5-nitrofur-2-carboxylic acid phenylamide; 5-nitrofur-2-carboxylic acid (furan-2-ylmethyl)-amide; 5-nitrofur-2-carboxylic acid (4-cyano-phenyl)-amide; 5-nitrofur-2-carboxylic acid 4-methoxy-benzylamide; 5-nitrofur-2-carboxylic acid 2-chlorobenzylamide; 5-nitrofur-2-carboxylic acid 2,4-dimethoxybenzylamide; 5-nitrofur-2-carboxylic acid 3,4-dimethoxybenzylamide; 5-nitrofur-2-carboxylic acid 3,4,5-trimethoxybenzylamide; 5-nitrofur-2-carboxylic acid (1,2,3,4-tetrahydronaphthalen-1-yl)-amide; 5-nitrofur-2-carboxylic acid indan-1-ylamide; 5-nitrofur-2-carboxylic acid phenethyl-amide; 5-nitrofur-2-carboxylic

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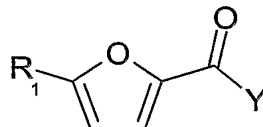
acid [2-(4-methoxy-phenyl)-ethyl]-amide; 5-nitrofur-2-carboxylic acid (1-phenyl-ethyl)-amide; 5-nitrofur-2-carboxylic acid [2-(3,4-dimethoxy-phenyl)-ethyl]-amide; 5-nitrofur-2-carboxylic acid 4-methoxy-benzylamide; 5-nitrofur-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-sulfo-fur-2-carboxylic acid; 5-(3-methoxy-phenylcarbonyl)-fur-sulfonic acid; 5-nitrofur-2-carboxylic acid (3-hydroxy-phenyl)-amide; 5-phenylsulfanyl-fur-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-benzyloxy-phenyl)-amide; 5-benzenesulfinyl-fur-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-benzenesulfonyl-fur-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid pyrazin-2-ylamide; 5-nitrofur-2-carboxylic acid (pyridin-2-yl methyl)-amide; 5-nitrofur-2-carboxylic acid (4-methoxy-benzothiazol-2-yl)-amide; 5-nitrofur-2-carboxylic acid (6-methoxy-pyrimin-4-yl)-amide; 5-nitrofur-2-carboxylic acid 2-methoxy-benzylamide; 5-nitrofur-2-carboxylic acid 2,3-dimethoxy-benzylamide; 5-nitrofur-2-carboxylic acid 4-thiomorpholin-4-yl-benzylamide; 5-nitrofur-2-carboxylic acid 4-(1-oxo-1,4-thiomorpholin-4-yl)-benzylamide; 5-nitrofur-2-carboxylic acid 4-(1,1-dioxo-1,4-thiomorpholin-4-yl)-benzylamide; 5-nitrofur-2-carboxylic acid 4-(3-amino-pyrrolidin-1-yl)-benzylamide; 4-(4-[(5-nitrofur-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid tert-butyl ester; 5-nitrofur-2-carboxylic acid 4-piperazin-1-yl-benzylamide; 5-nitrofur-2-carboxylic acid 4-(4-cyclopropylmethyl-piperazin-1-yl)-benzylamide; 5-nitrofur-2-carboxylic acid 4-(4-benzyl-piperazin-1-yl)-3-fluoro-benzylamide; 5-nitrofur-2-carboxylic acid 3-fluoro-4-(4-methyl-piperazin-1-yl)-benzylamide; 5-nitrofur-2-carboxylic acid 3-fluoro-4-thiomorpholin-4-yl-benzylamide; 5-nitrofur-2-carboxylic acid 3-fluoro-4-morpholin-4-yl-benzylamide; 5-nitrofur-2-carboxylic acid 4-(4-benzyl-piperidin-1-yl)-3-fluoro-benzylamide; 4-(4-[(5-nitrofur-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid ethyl ester; 4-(4-[(5-nitrofur-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic

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acid propylamide; and 4-(4-[(5-nitrofuranyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid isopropylamide.

- 5 44. The method of Claim 29, wherein the compound is selected from the group consisting of *N*-(4-methoxybenzyl)-5-nitrofuranyl-2-carboxamide; (3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl)(5-nitrofuranyl)methanone; *N*-((benzo[*d*][1,3]dioxol-6-yl)methyl)-5-nitrofuranyl-2-carboxamide; *N*-(2,4-dimethoxybenzyl)-5-nitrofuranyl-2-carboxamide; *N*-(3,4-dimethoxyphenethyl)-5-nitrofuranyl-2-carboxamide; *N*-(3,4-dimethoxybenzyl)-5-nitrofuranyl-2-carboxamide; and *N*-(4-methoxyphenyl)-5-nitrofuranyl-2-carboxamide.
- 10

- 15 45. A method of treating a microbial infection in a subject comprising administering to the subject a therapeutically effective amount of the compound having the following general formula:



wherein  $R_1$  is selected from the group consisting of halo, nitro, alkyl ester, phenylsulfanyl, phenylsulfinyl, phenylsulfonyl and sulfonic acid; and Y is a substituted amine.

- 20 46. The method of Claim 45, wherein the microbial infection is caused by a member of the genus *Mycobacterium*.
- 25 47. The method of Claim 46, wherein the member of the genus *Mycobacterium* is *Mycobacterium tuberculosis*.
48. The method of Claim 45, wherein the therapeutically effective amount is about 1 to about 1000 micromoles per milliliter.

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49. The method of Claim 48, wherein the therapeutically effective amount is about 10 to 500 micromoles per milliliter.

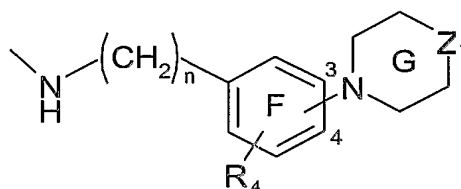
50. The method of Claim 49, wherein the therapeutically effective amount is about 50 to 250 micromoles per milliliter.

51. The method of Claim 50, wherein the therapeutically effective amount is about 100 to 200 micromoles per milliliter.

52. The method of Claim 45, wherein Y is selected from the group consisting of:

(a)  $-NR_2R_3$ , and  $R_2$  and  $R_3$  are each independently selected from the group consisting of H, alkyl, aryl, substituted alkyl, and substituted aryl, or  $R_2$  and  $R_3$  together form a ring structure with the nitrogen atom to which they are attached;

(b)



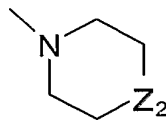
wherein  $n$  is an integer from 0 to 8;  $R_4$  is selected from the group consisting of hydrogen, halo, alkyl, substituted alkyl, and alkoxy; and  $Z_1$  is selected from the group consisting of oxygen, sulfur, sulfoxide,

sulfone,  $NR_5$ , and  $C(R_6)(R_7)$ ; wherein  $R_5$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkoxycarbonyl, aryl, substituted aryl, and  $-(C=O)-NR_8R_9$ , wherein  $R_6$  and  $R_7$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl and  $R_8$  and  $R_9$  are

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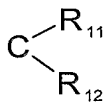
each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl;

(c)



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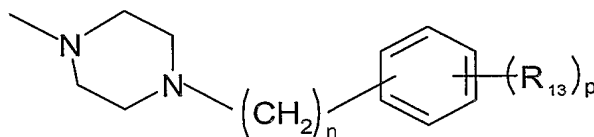
wherein  $Z_2$  is selected from the group consisting of oxygen,  $NR_{10}$ ,



and  $C(R_{11})(R_{12})$ ;  $R_{10}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl; and  $R_{11}$  and  $R_{12}$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl;

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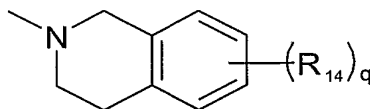
(d)



wherein  $n$  is an integer from 0 to 8;  $p$  is an integer from 1 to 5; and  $R_{13}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl;

15

(e)

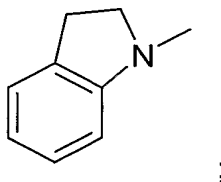


wherein  $q$  is an integer from 1 to 4; and  $R_{14}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl;

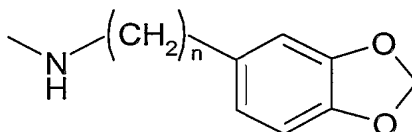
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(f)

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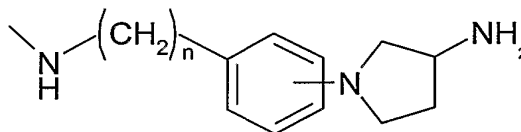


(g)



wherein n is an integer from 0 to 8; and

(h)



wherein n is an integer from 0 to 8.

53. The method of Claim 45, wherein Y is selected from the group consisting of anisidine, 3-halo-aniline, 3-anisidine, 4-anisidine, cyclohexylamine, adamantyl amine, furfuryl amine, 4-amino-benzonitrile, 4-methoxy-benzylamine, 2-chloro-benzylamine, 2,4-dimethoxy-benzylamine, 3,4-dimethoxy-benzylamine, 3,4,5-trimethoxy-benzylamine, 1-amino-1,2,3,4-tetrahydro-naphthalene, 1-amino-indane, phenethylamine, 4-ethoxy-phenethylamine, (S)-1-phenyl-ethylamine, (R)-1-phenyl-ethylamine, 3,4-dimethoxy-phenethylamine, 4-methoxy-benzylamine, 3-amino-phenol, 3-benzyloxy-aniline, N-methyl-aniline, N-methyl-4-anisidine, 2,3-dihydro-indole, 2-amino-pyridine, 3-amino-pyridine, 4-amino-pyridine, 3-amino-pyrazole, 2-amino-pyrazine, 2-amino methyl pyridine, 2-amino-4-methoxy-benzothiazole, 4-amino-6-methoxy-pyrimidine, 2-methoxy-benzylamine, and 2,3-dimethoxy-benzylamine.

54. The method of Claim 53, wherein Y is 3-chloro-aniline.



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55. The method of Claim 53, wherein Y is 3-fluoro-aniline.
56. The method of Claim 53, wherein Y is 3-anisidine.
57. The method of Claim 53, wherein Y is 4-methoxy-benzylamine.
58. The method of Claim 53, wherein Y is 3,4-dimethoxy-benzylamine.
59. The method of Claim 45, wherein the compound is selected from the group consisting of 5-nitrofur-2-carboxylic acid (3-chloro-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-bromo-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-fluoro-phenyl)-amide; 5-nitrofur-2-carboxylic acid (4-methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid adamantan-1-ylamide; 5-nitrofur-2-carboxylic acid phenylamide; 5-nitrofur-2-carboxylic acid (furan-2-ylmethyl)-amide; 5-nitrofur-2-carboxylic acid (4-cyano-phenyl)-amide; 5-nitrofur-2-carboxylic acid 4-methoxy-benzylamide; 5-nitrofur-2-carboxylic acid 2-chlorobenzylamide; 5-nitrofur-2-carboxylic acid 2,4-dimethoxybenzylamide; 5-nitrofur-2-carboxylic acid 3,4-dimethoxybenzylamide; 5-nitrofur-2-carboxylic acid 3,4,5-trimethoxybenzylamide; 5-nitrofur-2-carboxylic acid (1,2,3,4-tetrahydronaphthalen-1-yl)-amide; 5-nitrofur-2-carboxylic acid indan-1-ylamide; 5-nitrofur-2-carboxylic acid phenethyl-amide; 5-nitrofur-2-carboxylic acid [2-(4-methoxy-phenyl)-ethyl]-amide; 5-nitrofur-2-carboxylic acid (1-phenyl-ethyl)-amide; 5-nitrofur-2-carboxylic acid [2-(3,4-dimethoxy-phenyl)-ethyl]-amide; 5-nitrofur-2-carboxylic acid 4-methoxybenzylamide; 5-nitrofur-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-sulfo-furan-2-carboxylic acid; 5-(3-methoxy-phenylcarbonyl)-furan-sulfonic acid; 5-nitrofur-2-carboxylic acid (3-hydroxy-phenyl)-amide; 5-phenylsulfanyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-

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5 nitrofur-2-carboxylic acid (3-benzyloxy-phenyl)-amide; 5-benzenesulfinyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-benzenesulfonyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid pyrazin-2-ylamide; 5-nitrofur-2-carboxylic acid (pyridin-2-yl methyl)-amide; 5-nitrofur-2-carboxylic acid (4-methoxy-benzothiazol-2-yl)-amide; 5-nitrofur-2-carboxylic acid (6-methoxy-pyrimin-4-yl)-amide; 5-nitrofur-2-carboxylic acid 2-methoxy-benzylamide; 5-nitrofur-2-carboxylic acid 2,3-dimethoxy-benzylamide; 5-nitrofur-2-carboxylic acid 4-thiomorpholin-4-yl-benzylamide; 5-nitrofur-2-carboxylic acid 4-(1-oxo-114-thiomorpholin-4-yl)-benzylamide; 5-nitrofur-2-carboxylic acid 4-(1,1-dioxo-116-thiomorpholin-4-yl)-benzylamide; 5-nitrofur-2-carboxylic acid 4-(3-amino-pyrrolidin-1-yl)-benzylamide; 4-(4-[(5-nitrofur-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid tert-butyl ester; 5-nitrofur-2-carboxylic acid 4-piperazin-1-yl-benzylamide; 5-nitrofur-2-carboxylic acid 4-(4-cyclopropylmethyl-piperazin-1-yl)-benzylamide; 5-nitrofur-2-carboxylic acid 4-(4-benzyl-piperazin-1-yl)-3-fluoro-benzylamide; 5-nitrofur-2-carboxylic acid 3-fluoro-4-(4-methyl-piperazin-1-yl)-benzylamide; 5-nitrofur-2-carboxylic acid 3-fluoro-4-thiomorpholin-4-yl-benzylamide; 5-nitrofur-2-carboxylic acid 3-fluoro-4-morpholin-4-yl-benzylamide; 5-nitrofur-2-carboxylic acid 4-(4-benzyl-piperidin-1-yl)-3-fluoro-benzylamide; 4-(4-[(5-nitrofur-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid ethyl ester; 4-(4-[(5-nitrofur-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid propylamide; and 4-(4-[(5-nitrofur-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid isopropylamide.

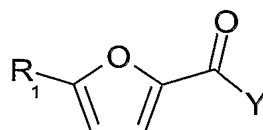
60. The method of Claim 45, wherein the compound is selected from the group consisting of *N*-(4-methoxybenzyl)-5-nitrofur-2-carboxamide; (3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl)(5-nitrofur-2-yl)methanone; *N*-((benzo[*d*][1,3]dioxol-6-yl)methyl)-5-nitrofur-2-

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carboxamide; *N*-(2,4-dimethoxybenzyl)-5-nitrofuran-2-carboxamide; *N*-(3,4-dimethoxyphenethyl)-5-nitrofuran-2-carboxamide; *N*-(3,4-dimethoxybenzyl)-5-nitrofuran-2-carboxamide; and *N*-(4-methoxyphenyl)-5-nitrofuran-2-carboxamide.

5

61. A pharmaceutical formulation for the treatment of tuberculosis, comprising a compound having the following general structure in a pharmaceutically acceptable carrier:



10

wherein  $R_1$  is selected from the group consisting of halo, nitro, alkyl ester, phenylsulfanyl, phenylsulfinyl, phenylsulfonyl and sulfonic acid; and Y is a substituted amine.

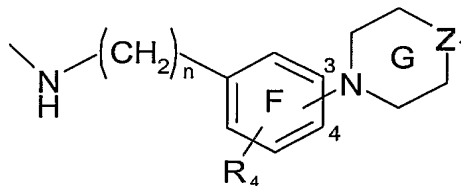
15

62. The pharmaceutical formulation of Claim 61, wherein Y is selected from the group consisting of:

(a)  $-NR_2R_3$ , and  $R_2$  and  $R_3$  are each independently selected from the group consisting of H, alkyl, aryl, substituted alkyl, and substituted aryl, or  $R_2$  and  $R_3$  together form a ring structure with the nitrogen atom to which they are attached;

20

(b)

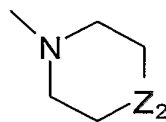


wherein n is an integer from 0 to 8;  $R_4$  is selected from the group consisting of hydrogen, halo, alkyl, substituted alkyl, and alkoxy; and  $Z_1$  is selected from the group consisting of oxygen, sulfur, sulfoxide,

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sulfone,  $\text{NR}_5$ , and  $\text{C} \begin{smallmatrix} \text{---} \text{R}_6 \\ \text{---} \text{R}_7 \end{smallmatrix}$ ; wherein  $\text{R}_5$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkoxy carbonyl, aryl, substituted aryl, and  $-(\text{C}=\text{O})-\text{NR}_8\text{R}_9$ , wherein  $\text{R}_6$  and  $\text{R}_7$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl and  $\text{R}_8$  and  $\text{R}_9$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl;

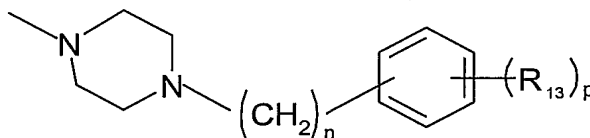
(c)



wherein  $\text{Z}_2$  is selected from the group consisting of oxygen,  $\text{NR}_{10}$ ,

and  $\text{C} \begin{smallmatrix} \text{---} \text{R}_{11} \\ \text{---} \text{R}_{12} \end{smallmatrix}$ ;  $\text{R}_{10}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl; and  $\text{R}_{11}$  and  $\text{R}_{12}$  are each independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and hydroxyl;

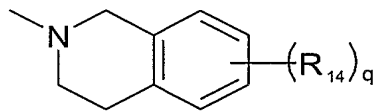
(d)



wherein  $n$  is an integer from 0 to 8;  $p$  is an integer from 1 to 5; and  $\text{R}_{13}$  is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl;

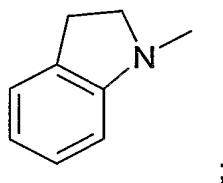
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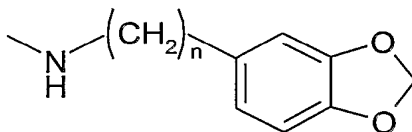


wherein q is an integer from 1 to 4; and R<sub>14</sub> is selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl;

(f)

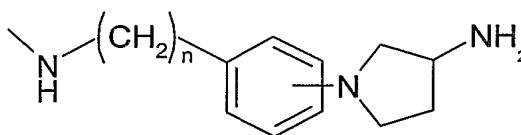


(g)



wherein n is an integer from 0 to 8; and

(h)



wherein n is an integer from 0 to 8.

63. The pharmaceutical formulation of Claim 61, wherein Y is selected from the group consisting of anisidine, 3-halo-aniline, 3-anisidine, 4-anisidine, cyclohexylamine, adamantyl amine, furfuryl amine, 4-amino-benzonitrile, 4-methoxy-benzylamine, 2-chloro-benzylamine, 2,4-dimethoxy-benzylamine, 3,4-dimethoxy-benzylamine, 3,4,5-trimethoxy-benzylamine, 1-amino-1,2,3,4-tetrahydro-naphthalene, 1-amino-indane,

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phenethylamine, 4-ethoxy-phenethylamine, (*S*)-1-phenyl-ethylamine, (*R*)-1-phenyl-ethylamine, 3, 4-dimethoxy-phenethylamine, 4-methoxy-benzylamine, 3-amino-phenol, 3-benzyloxy-aniline, N-methyl-aniline, N-methyl-4-anisidine, 2,3-dihydro-indole, 2-amino-pyridine, 3-amino-pyridine, 4-amino-pyridine, 3-amino-pyrazole, 2-amino-pyrazine, 2-amino methyl pyridine, 2-amino-4-methoxy-benzothiazole, 4-amino-6-methoxy-pyrimidine, 2-methoxy-benzylamine, and 2,3-dimethoxy-benzylamine.

64. The pharmaceutical formulation of Claim 63, wherein Y is 3-chloro-aniline.

65. The pharmaceutical formulation of Claim 63, wherein Y is 3-fluoro-aniline.

66. The pharmaceutical formulation of Claim 63, wherein Y is 3-anisidine.

67. The pharmaceutical formulation of Claim 63, wherein Y is 4-methoxy-benzylamine.

68. The pharmaceutical formulation of Claim 63, wherein Y is 3,4-dimethoxy-benzylamine.

69. The pharmaceutical formulation of Claim 61, wherein the compound is selected from the group consisting of 5-nitrofur-2-carboxylic acid (3-chloro-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-bromo-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-fluoro-phenyl)-amide; 5-nitrofur-2-carboxylic acid (4-methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid adamantan-1-ylamide; 5-nitrofur-2-carboxylic acid phenylamide; 5-nitrofur-2-carboxylic acid (furan-2-ylmethyl)-amide; 5-nitrofur-2-carboxylic acid (4-cyano-phenyl)-amide; 5-nitrofur-2-carboxylic acid 4-methoxy-benzylamide; 5-nitrofur-2-carboxylic acid 2-

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chlorobenzylamide; 5-nitrofur-2-carboxylic acid 2,4-dimethoxybenzylamide; 5-nitrofur-2-carboxylic acid 3,4-dimethoxybenzylamide; 5-nitrofur-2-carboxylic acid 3,4,5-trimethoxybenzylamide; 5-nitrofur-2-carboxylic acid (1,2,3,4-tetrahydronaphthalen-1-yl)-amide; 5-nitrofur-2-carboxylic acid indan-1-ylamide; 5-nitrofur-2-carboxylic acid phenethyl-amide; 5-nitrofur-2-carboxylic acid [2-(4-methoxy-phenyl)-ethyl]-amide; 5-nitrofur-2-carboxylic acid (1-phenyl-ethyl)-amide; 5-nitrofur-2-carboxylic acid [2-(3,4-dimethoxy-phenyl)-ethyl]-amide; 5-nitrofur-2-carboxylic acid 4-methoxybenzylamide; 5-nitrofur-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-sulfo-furan-2-carboxylic acid; 5-(3-methoxy-phenylcarbamoyl)-furan-sulfonic acid; 5-nitrofur-2-carboxylic acid (3-hydroxy-phenyl)-amide; 5-phenylsulfanyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid (3-benzyloxy-phenyl)-amide; 5-benzenesulfinyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-benzenesulfonyl-furan-2-carboxylic acid (3-methoxy-phenyl)-amide; 5-nitrofur-2-carboxylic acid pyrazin-2-ylamide; 5-nitrofur-2-carboxylic acid (pyridin-2-yl methyl)-amide; 5-nitrofur-2-carboxylic acid (4-methoxy-benzothiazol-2-yl)-amide; 5-nitrofur-2-carboxylic acid (6-methoxy-pyrimin-4-yl)-amide; 5-nitrofur-2-carboxylic acid 2-methoxybenzylamide; 5-nitrofur-2-carboxylic acid 2,3-dimethoxybenzylamide; 5-nitrofur-2-carboxylic acid 4-thiomorpholin-4-yl-benzylamide; 5-nitrofur-2-carboxylic acid 4-(1-oxo-1,4-thiomorpholin-4-yl)-benzylamide; 5-nitrofur-2-carboxylic acid 4-(1,1-dioxo-1,4-thiomorpholin-4-yl)-benzylamide; 5-nitrofur-2-carboxylic acid 4-(3-amino-pyrrolidin-1-yl)-benzylamide; 4-(4-[(5-nitrofur-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid tert-butyl ester; 5-nitrofur-2-carboxylic acid 4-piperazin-1-yl-benzylamide; 5-nitrofur-2-carboxylic acid 4-(4-cyclopropylmethyl-piperazin-1-yl)-benzylamide; 5-nitrofur-2-carboxylic acid 4-(4-benzyl-piperazin-1-yl)-3-fluorobenzylamide; 5-nitrofur-2-carboxylic acid 3-fluoro-4-(4-methyl-

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5 piperazin-1-yl)-benzylamide; 5-nitrofur-2-carboxylic acid 3-fluoro-4-thiomorpholin-4-yl-benzylamide; 5-nitrofur-2-carboxylic acid 3-fluoro-4-morpholin-4-yl-benzylamide; 5-nitrofur-2-carboxylic acid 4-(4-benzylpiperidin-1-yl)-3-fluoro-benzylamide; 4-(4-[(5-nitrofur-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid ethyl ester; 4-(4-[(5-nitrofur-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid propylamide; and 4-(4-[(5-nitrofur-2-carbonyl)-amino]-methyl)-phenyl)-piperazine-1-carboxylic acid isopropylamide.

10 70. The pharmaceutical formulation of Claim 61, wherein the compound is selected from the group consisting of *N*-(4-methoxybenzyl)-5-nitrofur-2-carboxamide; (3,4-dihydro-6,7-dimethoxyisoquinolin-2(1*H*)-yl)(5-nitrofur-2-yl)methanone; *N*-((benzo[*d*][1,3]dioxol-6-yl)methyl)-5-nitrofur-2-carboxamide; *N*-(2,4-dimethoxybenzyl)-5-nitrofur-2-carboxamide; *N*-(3,4-dimethoxyphenethyl)-5-nitrofur-2-carboxamide; *N*-(3,4-dimethoxybenzyl)-5-nitrofur-2-carboxamide; and *N*-(4-methoxyphenyl)-5-nitrofur-2-carboxamide.

20 71. The pharmaceutical formulation of Claim 61, wherein the formulation is acceptable for intravenous administration.

72. The pharmaceutical formulation of Claim 61, wherein the formulation is acceptable for oral administration.



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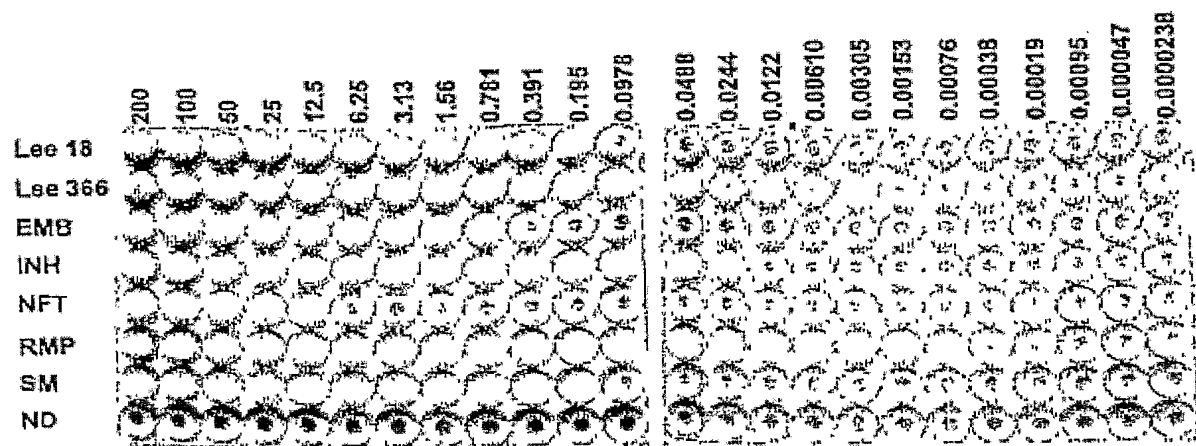
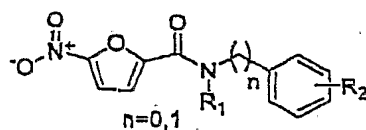
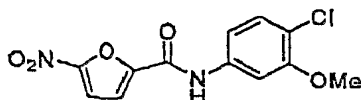


Figure 1

2/3



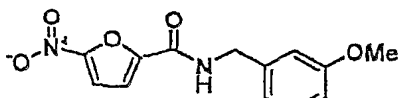
## Screening Hit



1st generation optimization library

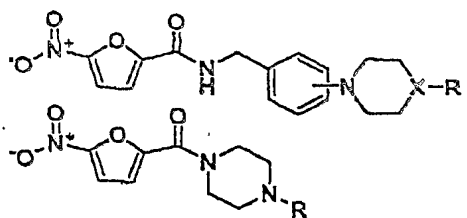


**1st generation lead**



Lee-18

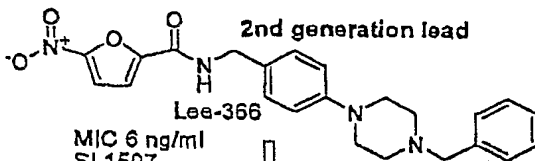
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SI 90.9



## 2nd generation optimization libraries

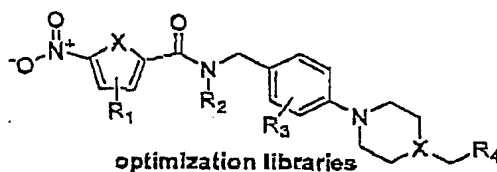


## 2nd generation lead



Lee-366

MIC 6 ng/ml  
SI 1597



## optimization libraries

### Figure 2

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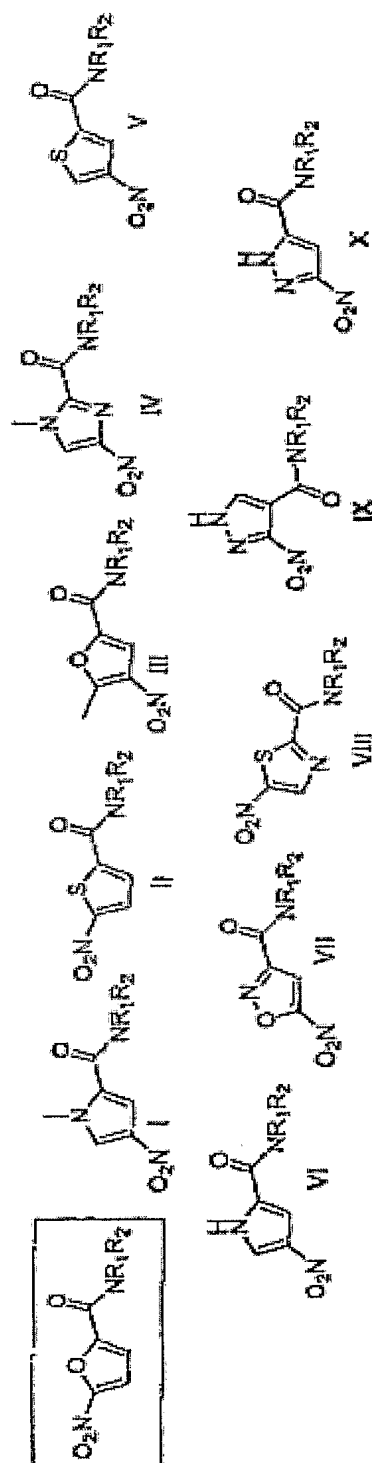


Figure 3

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31/4525, 31/496, C07D 307/70, 401/12, 403/12

(74) Agent: TAYLOR, Arles, A., Jr.; Jenkins, Wilson & Taylor, P.A., Suite 1400 University Tower, 3100 Tower Boulevard, Durham, NC 27707 (US).

(21) International Application Number:  
PCT/US2004/022491

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(71) Applicants (*for all designated States except US*): **THE UNIVERSITY OF TENNESSEE RESEARCH FOUNDATION** [US/US]; 1534 White Avenue-Suite 403, Knoxville, TN 37996-1527 (US). **COLORADO STATE UNIVERSITY RESEARCH FOUNDATION** [US/US]; P.O. Box 483, Fort Collins, CO 80522 (US).

**Published:**

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

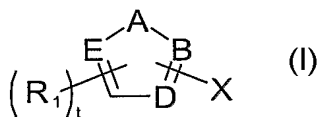
(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **LEE, Richard, E.** [US/US]; 8233 Byre Hollow Cave, Cordova, TN 38018 (US). **TANGALLAPALLY, Rajendra, Prasad** [IN/US]; Department of Pharmaceutical Science, 847 Monroe Ave - Room 327, Memphis, TN 38163 (US). **MCNEIL, Michael, R.** [US/US]; 6801 N County Road-15, Fort Collins, CO 80524 (US). **LENAERTS, Anne** [BE/US]; 4230 Goldeneye Drive, Fort Collins, CO 80526 (US).

(88) Date of publication of the international search report:  
29 December 2005

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: HETEROCYCLIC AMIDES WITH ANTI-TUBERCULOSIS ACTIVITY



(57) Abstract: Compounds having the general structure (I) : wherein A is selected from the group consisting of oxygen, sulfur, and NR<sub>15</sub>, and R<sub>15</sub> is selected from the group consisting of H, alkyl, aryl, substituted alkyl, and substituted aryl; B, D, and E are each independently selected from the group consisting of CH, nitrogen, sulfur and oxygen; R<sub>1</sub> is selected from the group consisting of nitro, halo, alkyl ester, phenylsulfanyl, phenylsulfinyl, phenylsulfonyl and sulfonic acid; t is an integer from

1 to 3; and X is a substituted amide and methods of using the novel compounds for treating infections caused microorganisms, including *Mycobacterium tuberculosis*.



WO 2005/007625 A3

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/22491

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/345, 31/4525, 31/496; C07D 307/70, 401/12, 403/12.

US CL : 514/254.1, 326, 472; 544/379; 546/214; 549/482..

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/254.1, 326, 472; 544/379; 546/214; 549/482..

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
CAS ONLINE STRUCTURE SEARCH

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/0119817 A1 (MEHTA et al.) 26 June 2003 (26.06.2003), see entire document but especially piperazine species on pages 4 and 7-12.	1-5,7,16,18,29-36,45-52,61-62,71-72
X	Chem.abstr., Vol.55, No.12, 12 June 1961 (Columbus, OH.,USA), columns 11386-11387, the abstract No. 11386i, FARCASAN V.'Substituted Anilides of 5-nitro-2-furoic acid' Rev.chim. Acad. rep. populaire Roumaine 1960 5, No.1,129-137 (German). See species listed in column 11387.	1-6,20, 21,23,27-29,32-38,40,43-45,48-54,56,59-64,66, 69-72

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

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Date of the actual completion of the international search

01 September 2005 (01.09.2005)

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, Virginia 22313-1450

Facsimile No. (571) 273-3201

Date of mailing of the international search report

27 OCT 2005

Authorized officer

Emily Bernhardt

Telephone No. (571) 272-2717

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/22491

### Continuation of Box II Reason 2:

The claims pertain to an extremely large number of permutations based on the scope of variables as generically set forth in the claims which are not all adequately supported in the description pages within the meaning of PCT Article 6. The claims have been searched based on representative examples set forth in claims 27-28,43-44,59-60 and 69-70. Additionally, it should be noted that the claimed subject matter lacks a significant structural element qualifying as the special technical feature that clearly defines a contribution over the art.